Improving the safety and quality of milk

Volume 1: Milk production and processing

Edited by Mansel W. Griffiths

CRC

WP

Improving the safety and quality of milk

Related titles:

Dairy processing: improving quality
(ISBN 978-1-85573-676-4)
With its distinguished international team of contributors, *Dairy processing* summarises key developments in the field and how they enhance dairy product safety and quality. The first part of the book discusses raw milk composition, production and quality. Part II reviews developments in processing from hygiene and HACCP systems to automation, high-pressure processing and modified atmosphere packaging. The final part considers developments for particular products such as fermented dairy products and cheeses.

Dairy-derived ingredients: food and nutraceutical uses
(ISBN 978-1-84569-465-4)
Advances in technologies for the extraction and modification of valuable milk components have opened up new opportunities for the food and nutraceutical industries. New applications for dairy ingredients are also being found. *Dairy-derived ingredients* reviews the latest developments in these dynamic areas. The first part covers modern approaches to the separation of dairy components and manufacture of dairy ingredients. The second part focuses on the biological functionality of dairy components and their nutraceutical applications. The final part of the book addresses the technological functionality of dairy components and their applications in food and non-food products.

Foodborne pathogens: hazards, risk analysis and control Second edition
(ISBN 978-1-84569-362-6)
Effective control of pathogens continues to be of great importance to the food industry. The first edition of *Foodborne pathogens* quickly established itself as an essential guide for all those involved in the management of microbiological hazards at any stage in the food production chain. This major new edition strengthens that reputation, with extensively revised and expanded coverage, including more than ten new chapters. Part I focuses on risk assessment and management in the food chain. Chapters in this section cover pathogen detection, microbial modelling, the risk assessment procedure, pathogen control in primary production, hygienic design and sanitation, among other topics. Parts II and III then review the management of key bacterial and non-bacterial foodborne pathogens.

Details of these books and a complete list of Woodhead titles can be obtained by:

- visiting our web site at www.woodheadpublishing.com
- contacting Customer Services (email: sales@woodheadpublishing.com; fax: +44 (0) 1223 893694; tel.: +44 (0) 1223 891358 ext. 130; address: Woodhead Publishing Limited, Abington Hall, Granta Park, Great Abington, Cambridge CB21 6AH, UK)

Improving the safety and quality of milk

Volume 1: Milk production and processing

Edited by
Mansel W. Griffiths

CRC

CRC Press
Boca Raton Boston New York Washington, DC

WOODHEAD PUBLISHING LIMITED
Oxford   Cambridge   New Delhi

Contents

Contributor contact details

(* = main contact)

Chapter 1

Professor A. L. Kelly*
Department of Food and Nutritional
    Sciences
University College Cork
Ireland
E-mail: a.kelly@ucc.ie

Dr L. B. Larsen
Department of Food Science
Faculty of Agricultural Sciences
University of Aarhus
Denmark
E-mail: lottebach.larsen@agrsci.dk

Chapter 2

Professor Mansel W. Griffiths
Department of Food Science and
    Canadian Research Institute for
    Food Safety
University of Guelph
Guelph
Ontario
Canada N1G 2W1
E-mail: mgriffit@uoguelph.ca

Chapter 3

Dr K. J. Burgess
Dairy Crest Technical Centre
Crudgington
Telford
Shropshire TF6 6HY
UK
E-mail:
    ken@kenburgessassociates.co.uk

Chapter 4

Dr B. Stessl and Dr I. Hein*
University of Veterinary Medicine
   Vienna
Department for Farm Animals and
   Veterinary Public Health
Institute for Milk Hygiene, Milk
   Technology and Food Science
Veterinaerplatz 1
A-1210 Vienna
Austria
E-mail:
   Ingeborg.Hein@vetmeduni.ac.at

Chapters 5 and 6

Dr Subir Kumar Nag
Division of Plant Animal
   Relationship
Indian Grassland and Fodder Research
   Institute
Jhansi-284 003 (UP)
India
E-mail: nagsk_67@rediffmail.com
   subirknag@yahoo.com

Chapter 7

M. C. te Giffel and
   Dr Ir. Marjon H.J. Wells-Bennik*
NIZO food research
Kernhemseweg 2
6718 ZB Ede
The Netherlands
E-mail: marjon.wells-bennik@nizo.nl

Chapter 8

Professor Johan A. M. van Arendonk*
   and Henk Bovenhuis
Animal Breeding and Genomics
   Centre
Wageningen University
PO Box 338
6700 AH Wageningen
The Netherlands
E-mail: Johan.vanArendonk@wur.nl

Hein J. F. van Valenberg
Dairy Science and Technology Group
Wageningen University
PO Box 338
6700 AH Wageningen
The Netherlands

Chapter 9

Professor O. M. Harstad*
Department of Animal and
   Aquacultural Sciences
Norwegian University of Life
   Sciences
P.O. Box 5003
1432 Aas
Norway
E-mail: odd.harstad@umb.no

Dr H. Steinshamn
Norwegian Institute for Agricultural
   and Environmental Research
Organic Food and Farming Division
Gunnars veg 6
6630 Tingvoll
Norway
E-mail:
   havard.steinshamn@bioforsk.no

Chapter 10

Professor Jörn Hamann
Institute for Food Quality and Food
    Safety
University of Veterinary Medicine
    Hannover Foundation
Bischofsholer Damm 15
30173 Hannover
Germany
E-mail: jha911@hamann-
    kronshagen.de

Chapter 11

O. Cerf*
Alfort Veterinary School
7 avenue du Général de Gaulle
FR-94700 Maisons-Alfort
France
E-mail: ocerf@vet-alfort.fr

J.-M. Gautier
Livestock Institute
Department of Husbandry Techniques
    and Quality
Division of Animal Welfare, Health,
    Hygiene and Traceability
BP 42118
FR-31321 Castanet-Tolosan Cedex
France

P. Parguel
Livestock Institute
Division of Regional Actions of
    Franche-Comté
Chambre d'agriculture – Valparc-
    Espace Valentin Est
FR-25048 Besançon Cedex
France

Chapter 12

M. J. Lewis
School of Chemistry, Food and
    Pharmacy
University of Reading
UK
E-mail: m.j.lewis@reading.ac.uk

Chapter 13

Dr H. C. Deeth
School of Land, Crop and Food
    Sciences
University of Queensland
Brisbane, Q 4072
Australia
E-mail: h.deeth@uq.edu.au

Chapter 14

Natalie Hotrum, Martijn Fox*, Hein
    van Lieverloo, Erik Smit and
Peter de Jong
NIZO food research
PO Box 20
6710 BA Ede
The Netherlands
E-mail: martijn.fox@nizo.nl

Maarten Schutyser
Wageningen University and Research
    Centre
Department of Food and
    Bioprocessing
PO Box 8129
6700 EV Wageningen
The Netherlands

Chapter 15

Dr G. Gésan-Guiziou
INRA-Agrocampus Ouest
UMR1253 Science et Technologie du
    Lait et de l'œuf
65 rue de Saint Brieuc
F-35042 Rennes
France
E-mail: genevieve.gesan-
    guiziou@rennes.inra.fr

Chapter 16

Dr Ir. Thom Huppertz
NIZO food research
PO Box 20
6710 BA, Ede
The Netherlands
E-mail: thom.huppertz@nizo.nl

Chapters 17 and 18

Dr Gustavo V. Barbosa-Cánovas* and
    Daniela Bermúdez-Aguirre
Center for Nonthermal Processing of
    Food
Department of Biological Systems
    Engineering
Washington State University
Pullman, WA 99164-6120
USA
E-mail: barbosa@wsu.edu

Chapter 19

S. C. Murphy
171 Stocking Hall
Cornell University
Ithaca, NY 14853
USA
E-mail: scm4@cornell.eud

Woodhead Publishing Series in Food Science, Technology and Nutrition

Preface

Since I first started my career in dairy research at the now defunct Hannah Research Institute in 1974, the dairy industry worldwide has faced many changes. With regard to food safety, it has witnessed the emergence of food-borne pathogens not previously associated with dairy products, such as *Listeria monocytogenes* and *Escherichia coli* O157:H7 along with the introduction of preventive food safety management systems (HACCP) to limit the impact of these pathogens. This year has seen the publication of the sequence of the entire cow genome, a feat that opens up innumerable possibilities. The use of this information will allow us, for example, to improve production costs through identification of traits related to feed conversion, to produce milk with specific characteristics and to impact animal welfare by selection of animals with increased disease resistance. These are just a few of the benefits the industry may reap. With more research it is also becoming apparent that milk and milk products are not the nutritional minefield that many would have us believe, in fact dairy products possess bioactive components that show substantial promise for health promotion.

It is the intent of this book to provide up-to-date coverage of several facets related to the production and processing of safe, wholesome and nutritious dairy products, not only from bovine milk but also from other domesticated ruminants. The first volume includes chapters related to milk safety and quality and focuses on the microbiological and chemical safety of raw milk and technologies for analyzing and processing milk. In the second volume, nutritional, sensory and sustainability issues are addressed as well as those associated with other milk-producing mammals and specific milk products.

I would like to thank all the contributing authors for their hard work and patience in waiting for edits. I would particularly like to acknowledge the

contribution of Dr Richard Robinson, who sadly died during the production of this book. Dr Robinson was well known by all in the dairy industry for his research and the many books he edited.

On a personal note I would like to thank my wife, Susan, for her understanding and support, my two daughters, Megan and Bethan, and their respective husbands, Darren and Eric, and my four grandchildren, Rhys, Emma, Sophie and Evan, for keeping me young at heart.

I would also like to thank Dairy Farmers of Ontario for all the support they have given me over the last 20 years.

To all the readers of this book, I hope you learn from it and that it makes you realize that the proper production and processing of milk is complex and is carried out by professional and dedicated farmers and processors.

1

Milk biochemistry

A. L. Kelly, University College Cork, Ireland and L. Bach Larsen, University of Aarhus, Denmark

Abstract: As milk is the key base raw material for all dairy products, the safety and quality of such products are heavily influenced by the characteristics of the milk. In this chapter, the key constituents of milk (fat, protein, salts, lactose and enzymes) and their properties are described, and the factors affecting the chemical composition and processing characteristics of milk, such as diet and lactation, are discussed in detail.

Key words: milk, composition, quality, processing.

1.1 Introduction

Milk is the fluid secreted by female mammals for the purpose of providing high-level nutrition to their offspring in the first days or weeks of life. Mankind has, for millennia, domesticated a small number of mammalian species, e.g., cows, goats, sheep and buffalo, for the purpose of producing their milk over an artificially lengthened season, and consuming it either directly or after conversion into a range of dairy products. Today, a very significant proportion of food consumed worldwide has its origins in mammalian milk, and a huge and diverse dairy industry is at the forefront of the global food industry in terms of scale, economic significance and technological sophistication.

It is well known that many people worldwide, e.g. in Asia, have problems tolerating milk due to lactose intolerance. It has recently been discovered that the ability of most European adults to tolerate milk is the result of a mutation in a single gene, which gave our ancestors bearing that mutation an advantage for survival, and furthermore that the tolerance for milk in the Saudi population is

the result of a different mutation leading to the same adaptation to consumption of milk (Enattah *et al.*, 2008). The mutation results in continuous production of the enzyme responsible for the cleavage of lactose, the lactase enzyme (*β*-galactosidase), which is produced by cells in the intestine. This mutation is thought to have originated in the Caucasus region before people migrated to Europe after the last ice age. Even though it is sometimes said by some people that 'milk is not intended as food for adults', the discovery of this mutation, which is thought to have its origin in a single person from whom it was spread, strongly indicated this to be very beneficial for the survival of our ancestors. During the last ice age, it gave them the possibility of exploiting the valuable nutrients in milk from domesticated animals, which was an obvious advantage for their survival at times with limited food alternatives.

The characteristics and quality of dairy products from market milk to cheese and yoghurt depend to a large extent on the primary stage of milk secretion within the mammal, and milk is a highly variable and complex raw material for processing. Hence, understanding of the mechanism of secretion of milk, the factors affecting the composition of milk, and ways in which milk composition and yield can be manipulated are of great interest to processors and farmers alike.

The objective of this chapter is to outline the major constituents of milk and their properties, to explore the manner in which they are secreted in the mammal, and to discuss factors affecting this production, and hence the quality of milk. The focus of the discussion will be on bovine milk, as the predominant milk-supplying species in most countries.

1.2   Milk composition and constituents

Milk is an enormously complex physicochemical system, with multiple constituents in different phases and states existing in a delicate balance of forces which exists on the brink of stability. It can readily be destabilised so as to collapse into separated or altered states; indeed, these phenomena had been exploited to produce dairy products long before their scientific mechanisms were understood.

In essence, milk is a solution of dilute salts, a simple sugar and vitamins, in which fat is emulsified as globules, and which contains a complex system of proteins, most of which exist in colloidal aggregates of thousands of molecules (casein micelles), an order of magnitude smaller than the fat globules. Studying milk under progressively higher microscopic magnification thus reveals a teeming multiphase system of complex biological molecules arranged in highly structured complex entities.

1.2.1   Lactose
In concentration terms, the dominant constituent of milk is generally lactose, a disaccharide consisting of one linked molecule each of glucose and galactose,

which is present at 4.5–5.0% in bovine milk. The level of lactose in milk is relatively constant, and has an influential role on milk yield, as lactose is synthesised by the mammary gland, and determines how much water is drawn into the milk. The presence of lactose makes milk a highly fermentable medium, as a large number of bacterial species (collectively termed the lactic acid bacteria) can hydrolyse lactose to lactic acid, which reduces the pH of milk and, as we will see, results in coagulation if this drop is great enough (i.e., when the pH reaches 4.6, the isoelectric point of casein). While uncontrolled or unwanted fermentation clearly results in spoilage of the milk, controlling this fermentation is the basis of production of dairy products such as cheese and yoghurt. Lactose is also of interest due to its propensity, as a reducing sugar, to undergo Maillard reactions at high temperature, leading to colour changes in milk heated to very high temperatures (e.g., during sterilisation processes), and to its crystallisation behaviour, which is principally of significance in highly concentrated dairy systems, such as evaporated milk.

1.2.2   Milk fat

The next most abundant substance in milk is generally fat, although the level of fat can vary from below 3.0% to more than 5.0%, a much greater range than that of any milk constituent. The main constituent of milk fat is triglycerides (more than 95% of the milk fat), which consist of three fatty acid molecules esterified to a glycerol molecule. Milk contains several types of fatty acids, differing in the length of the chain of carbon atoms (and classified on this basis into short-, medium- and long-chain) and numbers of double bonds, i.e., whether saturated or unsaturated (Jensen, 2002; Huppertz *et al.*, 2008). Compared to other types of food, milk fat is characterised by a great diversity of fatty acids, with chain lengths from four carbons up to more than 20 carbons, as well as branched fatty acids produced by microbes. The chemical properties of fatty acids have considerable consequences for both the nutritional quality of milk (in terms of the healthiness or otherwise of saturated fats) and its technological properties; chain length and degree of saturation both influence the melting point of the triglyceride, and hence the ultimate hardness of milk fat at, for example, refrigeration temperature. Compared to bovine milk fat, vegetable fats such as olive oil have a far higher proportion of unsaturated fatty acids, and hence provide both softness and perceived health benefits to consumers when added to products such as margarine and dairy spreads.

Milk fat also contains low levels of mono- and diglycerides, and minor constituents such as cholesterol, sphingolipids and phospholipids. Recently, attention has been drawn to some possible beneficial effects of some of the fatty acids in milk, including conjugated linoleic acid (CLA) and short-chain fatty acids (SCFAs) (Collomb *et al.*, 2006; McIntosh *et al.*, 2006; Bisig *et al.*, 2007; Chilliard *et al.*, 2007).

Milk fat is present in the milk as milk fat globules (MFG) with diameters ranging from 0.1 to more than 10 μm. The globules contain a nonpolar core of

triglycerides and cholesterol esters. The milk fat in the core is protected and rendered (almost) stable in the aqueous environment of milk by the presence of a protective coating on the surface of the spherical globules, the milk fat globule membrane (MFGM), which stabilises the emulsion and protects the triglycerides from degradation (lipolysis). The structure and composition of milk fat were reviewed by Jensen (2002), and the physical stability of milk fat globules was reviewed by Huppertz and Kelly (2006). The MFGM consists of a double-layer phospholipid membrane into which different proteins are embedded, giving the MFGM specific characteristics. These proteins include some major proteins such as butyrophilin and the enzyme xanthine oxidase, and an increasing list of minor proteins are associated with the MFGM (Reinhardt and Lippolis, 2006; Fong *et al.*, 2007). Studies on knock-out mice, in which functional genes coding for either xanthine oxidase or butyrophilin were lacking, have indicated some functions of the proteins associated with the MFGM. In both types of mice, large droplets of lipid without a proper outer membrane were secreted, and fused together into large aggregates of lipid; this strongly indicates that both of these proteins are essential for the production of intact milk fat droplets (Bauman *et al.*, 2006; Huppertz and Kelly, 2006).

The size of most of the milk fat droplets (more than 80% in number) is below $1\,\mu m$, but in terms of volume most of the milk fat is made up of fat globules with average diameter of approximately $4\,\mu m$. The stability of the emulsion is challenged primarily by the density difference between milk fat and the sur-rounding aqueous serum; this leads to relatively rapid separation of unprocessed milk into a phase enriched in milk fat globules in much closer contact with each other (i.e., cream) and a phase largely depleted of such globules (i.e., skimmed milk). On processing of milk, this separation can be accelerated (by applying centrifugal force) or hugely retarded (by reducing the size of the fat globules, using homogenisation, which greatly slows their rate of separation, as described by Stokes's Law). The globules do generally remain discrete, however, unless the integrity of the MFGM is severely compromised, either accidentally (through excessive agitation or pumping) or deliberately (in making butter, when the damaged globules are worked together into a homogeneous mass, from which most of the aqueous phase is drained).

The phospholipids mainly found at the outer side of the globules are more unsaturated than the triglycerides in the core of the fat globules (Jensen, 2002). Due to this, the level of unsaturated phospholipids is higher in milk with smaller fat globules. The levels of both phospholipids and triglycerides are affected by feeding (as will be discussed later).

Oxidation of lipids in milk is potentially a substantial problem for both milk and processed dairy products, as it gives rise to off-flavours and can reduce nutritional quality. The oxidative stability of milk is determined by a delicate balance between pro- and antioxidants in milk, where the concentration of polyunsaturated fatty acids, which are prone to oxidation, is believed to be an important factor for the stability (Barrefors *et al.*, 1995). Oxidation is often measured during storage of milk but, in several cases, oxidation has been

detected directly after milking, a phenomenon called spontaneous oxidation, where imbalance between pro- and antioxidants seems important for the development of off-flavour. Auto-oxidation is believed to be a major reason for the propagation of oxidation in milk during storage, and the concentrations of transition metal ions (Cu^+, Fe^{2+}) in milk play an important role as pro-oxidants (Ford *et al.*, 1986).

Light-induced oxidation is another major reason for off-flavours in dairy products. Milk contains a relatively high concentration of riboflavin, which can act as a photo-sensitiser in two ways: it can either directly oxidise proteins and lipids, or it can react with oxygen, forming the reactive oxygen species, singlet oxygen, which can further oxidise lipids. Milk also contains a range of potential antioxidants, such as tocopherols, carotenoids and uric acid (Ostdal *et al.*, 2000) formed by ruminal metabolism. However, there is an ongoing discussion of the significance of the antioxidants in relation to protection of milk from oxidation.

1.2.3   Proteins in milk

The proteins of milk are classed into two major groups, which differ fundamentally in their properties, in particular their solubility when the pH of milk is adjusted to 4.6. Under such conditions, the majority (typically around 75% by weight) of milk protein, called casein, is insoluble and either precipitates or forms a gel, depending on whether the rate of pH drop is rapid or slow, respectively. This casein fraction actually comprises four major caseins, called α_{s1}-, α_{s2}-, β- and κ-caseins, which are all relatively hydrophobic fibrous proteins, with little tertiary structure. The caseins associate in the aqueous environment of milk into exquisitely complex structures called micelles, containing thousands of molecules of each casein (for a review, see Fox and Kelly, 2004). The caseins are found in dairy cattle in different genetic variants (see Table 1.1 and Section 1.5.4). The caseins are phosphorylated to different extents and, in the case of κ-casein, may be glycosylated to different extents with carbohydrate groups including galactosamine, galactose and *N*-acetyl-nuraminic acid (Table 1.1). The variations in the amino acid sequences of the different genetic variants give the variant caseins slightly different molecular masses and, in some cases, also different isoelectric points (pI values). This is seen, for example, in κ-casein, where one of the differences between the variants is that the A variant has an aspartic acid residue at position 148, while variant B has an asparagine residue at that position. The presence of an extra aspartic acid provides an extra negative charge of the A variant compared with the B variant at the pH of milk, i.e. pH 6.7, and thereby some different processing characteristics of κ-casein type B milk compared with type A milk. This introduces, together with phosphorylation, further variations in the pI values of the different variants.

Of the four caseins, only α_{s2}- and κ-casein contain cysteine residues in their primary structure. Both molecules contain two cysteine residues per monomer. Due to the formation of disulphide bonds, the α_{s2}-casein molecules in milk are

Table 1.1   Genetic variants and some molecular characteristics of the caseins

Casein	Molecular mass[a] (kDa)	pI	Disulphides	Carbohydrate
α_{s1}-casein			No	No
A-8P	22.077	4.94		
B-8P	23.623	4.94		
C-8P	23.551	4.97		
D-9P	23.734	4.88		
α_{s2}-casein			Yes	No
A-10P	25.157	5.45		
A-11P	25.238	5.37		
A-12P	25.319	5.30		
A-13P	25.400	5.23		
β-casein			No	No
A^1-5P	24.028	5.22		
A^2-5P	23.988	5.14		
A^3-5P	23.979	5.07		
B-5P	24.097	5.29		
C-4P	23.946	5.46		
κ-casein			Yes	Maybe
A-1P	19.038	5.61		
A-2P	19.099	5.34		
B-1P	19.006	5.90		
B-2P	19.067	5.58		

[a] Mass of monomer.
Source: Data are according to Swaisgood (1992) and Holland *et al.* (2004). The pI values for A-2P and B-2P were calculated using the bioinformatics package at http://www.expasy.ch/ for the κ-casein sequence without the signal peptide.

found both as monomers and as dimers, linked together by two disulphide bridges. In contrast, κ-casein ranges in size from the monomer state to larger than a decamer, held together by an apparently randomised pattern of disulphide bonds between the cysteines (Rasmussen *et al.*, 1999). In the monomers, the cysteines are occupied in intra-chain disulphide bridges.

Over the last decades, several different models for the inner structure of the casein micelle have been proposed, but it seems that the micelles are composed of a tangled mass of protein molecules, interacting by crosslinks either between hydrophobic regions of the casein molecules or through calcium bridges (De Kruif and Holt, 2003; Horne, 2003). A key feature shared by all models is that the κ-casein is mainly found at the surface of the micelles; this is where it exerts a protective force due to its glycosylated nature, which lends it an amphiphilic character and the ability to stabilise the hydrophobic micelle core in a manner analogous to that in which the MFGM stabilises the fat globules. κ-Casein causes electrostatic and steric repulsion between micelles and, if this influence is negated, either by reducing the pH to the isoelectric point of casein (the afore-

mentioned value of 4.6) or by enzymatically removing the stabilising glyco-sylated part of the molecule (which is what the enzyme chymosin in rennet does during cheese-making), the unstable molecules aggregate into complex three-dimensional structures, to yield the gels that give yoghurt and cheese their structure. Thus, the properties of the casein micelle, and the means by which it may be physicochemically destabilised, are key to the manufacture of a range of dairy products.

The proteins which remain stable at pH 4.6 are termed the whey proteins, and are a family of globular proteins. The principal whey proteins by weight are β-lactoglobulin and α-lactalbumin, while minor whey proteins include serum albumin, immunoglobulins, lactoperoxidase and lactoferrin, in addition to a growing list of further proteins identified in milk by new proteomic techniques (Fong *et al.*, 2008). The main significance of the whey proteins, and β-lactoglobulin in particular, is that they unfold on heating and can subsequently interact and form complexes with themselves or other proteins. This is prin-cipally driven by the heat-induced exposure of a highly reactive free sulphydryl group in β-lactoglobulin, which can form disulphide bonds with any milk proteins containing disulphide bonds. Such reactions can lead to gel formation at sufficiently high whey protein concentrations (which is exploited during their use as food ingredients) and may be involved in phenomena such as the coagulation of milk on sterilisation, and the tendency of UHT-treated milk to gel during storage. In recent years there has been increasing focus on health-promoting proteins in milk, and on bioactive peptides derived by proteolytic cleavage, especially from the caseins.

1.2.4   Milk salts

Milk contains a wide range of mineral salts, some of which are associated with (and play a key role in maintaining the structure of) casein micelles, and some of which are in the serum phase of milk, in either ionised or non-ionised form; principal anionic salts in milk, in order of level, are potassium, calcium, sodium and magnesium, while principal cations include chloride, sulphate, carbonate, phosphate and citrate. The fraction of milk salts associated with the casein micelles is called colloidal calcium phosphate (CCP) and includes, as the name suggests, mainly calcium and phosphorus, but with lower levels of other species such as magnesium and citrate. The salt balance in milk between different forms and fractions is sensitive to processing conditions, in particular temperature and pH; reducing pH solubilises CCP progressively, while heating reduces the solubility of calcium and increases the CCP content of micelles.

Due to the content of lactose and salts in milk, the freezing point of milk is lower than that of water, milk with a freezing point between -0.516 and -0.545°C being considered normal. For example, a survey determined the mean value in Swedish dairy milk to be -0.529°C (Lindmark-Månsson *et al.*, 2003). Changes in the levels of lactose in milk will be counteracted by changes in milk salts, and vice versa, in order to maintain constant osmotic pressure. Taken

together, lactose, Cl^-, K^+ and Na^+ contribute 80% of the freezing point depression of milk. The freezing point of milk is measured regularly by dairies and is used as a test for water addition, which would raise the freezing point.

1.3   Indigenous enzymes in milk

Milk of all species contains a heterogeneous population of enzymes of different types and activities; bovine milk probably contains almost 70 enzymes, of which around 20 have been investigated in some detail. Recent reviews on the enzymology of milk include those by Fox and Kelly (2006a,b) and Kelly and Fox (2006).

   The four most important enzyme systems in milk, and their significance for product quality, will now be described.

1.3.1   Alkaline phosphatase

Alkaline phosphatase is arguably the best-known enzyme in milk, although its actual action in milk is probably of very little significance for milk quality. The reason for its importance lies rather in the serendipitous similarity of its thermal inactivation kinetics to those of the pathogenic bacterium *Mycobacterium tuberculosis*, and its ease of determination using assays based on colorimetric or fluorimetric substrates. As conventional pasteurisation is achieved using conditions designed to inactivate *M. tuberculosis* (heating at 72ºC for 15 seconds), rapid determination of the presence or absence of activity of alkaline phosphatase is widely used as an indicator of the effectiveness of pasteurisation (Fox and Kelly, 2006b).

1.3.2   Plasmin

In blood, an enzyme called plasmin plays a key role in the process of control of blood clotting, and the activity must thus be tightly regulated, which is achieved by a system including the inactive precursor of the enzyme (plasminogen), a set of activators of plasminogen (grouped into tissue- and urokinase-type activators), and inhibitors of both plasmin and its activators. The entire blood system appears to also exist in milk, and levels of all components probably increase in circumstances where influx of blood constituents and somatic cells in milk increase (e.g., mastitis). Plasmin, a serine proteinase with a pH optimum of 7.5, is probably the principal proteolytic enzyme in milk from healthy cows. The total concentration of plasmin and plasminogen is in the range 1–3 μg/ml, of which approximately 10% is active plasmin (Richardson and Pearce, 1981; Benfeldt *et al.*, 1994). Plasmin in milk can degrade the proteins in the milk through cleavage at bonds involving lysine or arginine residues in the polypeptide chains. Its significance arises from its hydrolysis of the caseins to yield the fractions called the γ-caseins and proteose peptones (fragments of β-

casein) and λ-caseins (fragments of α_{s1}-casein). Plasmin itself is relatively heat-stable, with low activity being detected even in UHT-treated milk (heated at 135–140ºC for 3–4 seconds), partially due to activation of residual plasminogen by heat-stable plasminogen activators (PA) (Enright *et al.*, 1999). In fact, the inhibitors of plasmin and plasminogen are probably more heat-labile than the enzyme or the activators, which can result in pasteurisation-like treatments actually increasing the net plasmin activity in milk, and consequent increases in proteolysis of casein (Richardson, 1983). The plasmin/plasminogen system in milk has been extensively studied, and has been shown to play a role in ripening of many cheese varieties (through initial or primary proteolysis of caseins to polypeptides which can be acted on by starter bacterial proteinases) and possibly also in gelation of UHT milk on storage (Kohlmann *et al.*, 1991; Bastian and Brown, 1996; Kelly *et al.*, 2006).

In raw milk, a high level of plasmin activity is normally not desirable, as it will result in a lower content of intact protein and, for example, can result in a lower cheese yield (Mara *et al.*, 1998). In cheese, however, plasmin activity contributes positively to cheese ripening through initial proteolysis of the caseins, on which the microbial proteases can subsequently act, and furthermore may influence the taste and texture of many cheese varieties, although perhaps to a relatively minor extent (Farkye and Fox, 1992).

1.3.3 Somatic cell proteinases

Milk contains a variable number of somatic cells (white blood cells) and the number (somatic cell count, SCC) and types of cell present depend on a number of factors, principally the presence of infection such as mastitis (see Section 1.5.6). Several proteolytic enzymes and enzyme activities have been suggested as being associated with somatic cells in milk. The release of these enzymes from somatic cells can result from either active secretion or release from damaged cells. The final definitive proof that these enzymes are derived from somatic cells has not yet been provided, only that their presence and derived activities to some extent correlate with SCC. It is actually an alternative possibility that at least some of these proteases are secreted by mammary epithelial cells; this is an issue that awaits further research (Kelly *et al.*, 2006).

Different cell types may have different enzyme profiles, and thus the enzyme profile of milk may be affected by both total SCC and also differential SCC (e.g., proportion of polymorphonuclear leucocytes, PMN, versus macrophages). For example, the proteases present during acute mastitis, where a large number of PMN are present in milk, may differ from the protease profile observed during chronic mastitis, where the majority of cells are macrophages.

The first so-called somatic cell protease suggested to be present in bovine milk was the lysosomal aspartic protease cathepsin D. It was later shown that the major part of the aspartic acid protease activity present in milk was derived from procathepsin D, and not from mature cathepsin D (Larsen and Petersen, 1995). At acid pH, at least at pH 3.5–5.0, milk procathepsin D can autoactivate into a

proteolytically active intermediate form, called pseudocathepsin D (Larsen *et al.*, 1993). As the pH of many cheeses is in the region of 5, it is interesting that cathepsin D and pseudocathepsin D can degrade the caseins into definite fragments, much like chymosin, and, furthermore, when added to milk in sufficient amounts, are actually able to coagulate milk (McSweeney *et al.*, 1995; Larsen *et al.*, 1996). Cathepsin D has been found to be more heat-stable in milk than in buffer, and approximately half of the activity derived from cathepsin D and procathepsin D in milk survived HTST pasteurisation at 72ºC (Larsen *et al.*, 2000; Hayes *et al.*, 2001). As procathepsin D and cathepsin D in milk are mainly associated with the whey fraction, their main significance in relation to quality of dairy products is potentially in ultrafiltrated (UF) rennet-free cheeses, like UF-feta, quarg and cottage cheese. They may also be active in some Swiss-type cheeses, where the added rennet has been heat-inactivated, in addition to whey powders and potentially some fermented products. Indeed, activity derived from cathepsin D has been detected both in quarg and in extracts of UF-feta (Hurley *et al.*, 2000; Larsen *et al.*, 2000).

Cysteine protease activity has also been detected in bovine mik (Magboul *et al.*, 2001) and different types of cysteine proteases have been fractionated. A partially purified fraction retained cysteine protease activity after heating at 72ºC for 30 seconds, and immunoblotting revealed the presence of immuno-reactive cathepsin B. It is, however, likely that other types of cysteine proteases, apart from cathepsin B, are present in milk, due both to the heterogeneous nature of partly purified fractions, and to the fact that other types of cysteine proteases are present in the bovine lysosomes. The distribution between mature forms of cathepsin B and eventual pro-forms of the enzyme in milk also remains to be established. Like cathepsin D, cathepsin B is also able to hydrolyse the caseins, but the specificity is different (Considine *et al.*, 2004).

It is very likely that other somatic cell proteinases remain to be identified in milk in the future. Some activities derived from unidentified milk proteases have been described (Larsen *et al.*, 2006), potential candidates for which include the serine proteinases neutrophil elastase and cathepsin G, but other proteinases may also be present, e.g. metalloproteinases. Some new techniques, including mass spectro-metry, have been employed recently for the detailed characterisation of the peptide profile in different types of high-cell-count milk and of healthy milk aiming at identifying responsible proteinases through the determination of cleavage sites (Wedholm *et al.*, 2008), which are to a large extent enzyme-specific.

1.3.4   Lipoprotein lipase

Lipoprotein lipase (LPL) is the enzyme in milk responsible for enzymatic lipolysis, i.e., the hydrolysis of fatty acids from triglycerides and phospholipids in the milk; LPL is also involved in the biosynthesis of milk fat (Huppertz *et al.*, 2008). Above a certain threshold, the released fatty acids can result in rancid off-flavours from short-chain fatty acids or from their oxidation to ketones. As long as the MFGM is intact, LPL cannot come into contact with its substrate,

especially the triglycerides; as a result of this highly efficient partitioning, the extent of lipolysis of milk is a fraction of what it should theoretically be. However, when the MFGM is damaged, e.g. by excessive pumping of raw milk, especially uncooled milk, or homogenisation of raw milk, the triglycerides are rendered susceptible to lipolysis, resulting in an increase in the free fatty acid level (Wiking *et al.*, 2003, 2005). In addition, some milk samples can undergo spontaneous lipolysis upon cooling of fresh, raw milk, which may depend on a balance of activating substances (e.g., apolipoproteins) and inhibiting substances (some proteins and peptides) present in milk. LPL in milk is reduced by pasteurisation, but more complete inactivation requires more severe heat treatments, as would be used for the processing of cream to be used in products where unwanted lipolysis could cause quality problems. The literature on lipoprotein lipases in milk was recently reviewed by Deeth (2006).

1.4   The secretion of milk

The production of milk involves a huge commitment of resources and energy by the mammal, and is mediated by the transport of raw materials from the blood into the udder, where the barrier between milk and blood is sufficiently porous to allow some constituents (lactose, minerals, enzymes, somatic cells) to transit in either direction. Other raw materials enter the mammary secretory cells for conversion and packaging into milk constituents and structures (e.g., fat globules, casein micelles), which then enter the milk.

The milk triglycerides are synthesised in the rough endoplasmic reticulum (ER) of the mammary cells. Small lipid droplets are released from the rough ER into the cytoplasm, where the lipid, coated with a bilayer membrane from the ER, becomes further coated with protein. Some of the droplets fuse with each other to form larger droplets on their way to the apical membrane of the cells (pathway A in Fig. 1.1), while others are secreted without fusion (pathway B in Fig. 1.1). When the lipid droplets arrive at the apical membrane, they are budded from the cell membrane ('blebbing'), by which process they receive the second membrane bilayer. Interestingly, the enzyme xanthine oxidase plays a key role in the secretion of milk fat globules, but in this context does not rely on its enzymatic activity (Harrison, 2006).

Most of the milk proteins, i.e., the caseins and most of the whey proteins, are also synthesised in the rough ER, then transported to the Golgi apparatus, where post-translational modifications, such as phosphorylation and glycosylation, occur. The modified proteins are released in secretory vesicles, and move to and fuse with the apical membrane, whereby the content is released into milk by exocytosis (pathway C in Fig. 1.1).

The primary physical location of milk production is an alveolus, which is a small bud-shaped chamber lined internally with the secretory mammary cells, and externally with a network of capillaries and muscle cells; the capillaries transport blood and precursors to the udder for milk production. The alveoli then

Fig. 1.1 The pathways for secretion of major milk constituents in mammary epithelial cells. A: The cytoplasmic lipid droplet pathway. B: The microlipid droplet pathway. C: The secretory pathway for proteins and salts. MFGM: Milk fat globule membrane. ER: Endoplasmic reticulum. Adapted from Mather and Keenan (1998) and Bauman *et al.* (2006).

drain into a system of ducts, each of which serves multiple alveoli, and which transport the freshly secreted milk into the central chamber or cistern for each quarter of the udder. When a cow is milked, either by hand or mechanically, electrical signals to the brain result in release of the hormone oxytocin, which induces contraction of the muscular scaffolding of the alveoli, squeezing out milk and resulting in a major increase in pressure within the quarter, which overcomes the resistance of the sphincter, which normally keeps the teat canal shut, and milk is ejected.

Clearly, milk production does not continue after milking at a constant rate, but rather the rate of secretion slows as the udder spaces fill, pressure builds up, and eventually milk production essentially ceases, generally in time for the next milking event. The agent which causes the cessation of milk production (the so-called feedback inhibitor of lactation) has been the subject of intense discussion and controversy for many years. Interestingly, recent studies have suggested that the agent which controls lactation may be a product of the hydrolysis of β-casein by plasmin (fragment 1-28), which is a potent blocker of potassium channels in apical membranes of mammary epethelia; injection of a solution containing this peptide in purified form results in a transient reduction in milk production (Silanikove *et al.*, 2000, 2006).

The application of hormones such as somatotrophin can increase milk yield significantly, but typically does not affect the gross composition of milk from

cows in positive energy balance; synthesis of milk constituents is increased proportionately, so that milk composition remains unaltered (Capuco and Akers, 2002). Increasing the frequency of milking (e.g. from two to three milkings daily) also increases total milk yield, and reducing to once-daily milking has the opposite effect (Lacy-Hulbert *et al.*, 1999).

1.5   Factors affecting milk composition and processing properties

Milk composition is not constant, which leads to significant challenges for manufacturers of dairy products. The biological mechanisms outlined above that result in the secretion of milk are sensitive to a number of physiological influences resulting from the status of the producing animals, and hence the composition of milk is likewise subject to significant influences relating to overall status of the producer. These influences will be considered in turn in this section.

1.5.1   Stage of lactation

The primary influence on milk composition and processing properties is probably the lactational status of the animal, as milk production is initiated by a specific incident, the birth of the neonate, and the secretion mechanism is regulated to provide the needs of such newborns at different times after birth. Hence, the composition of milk varies with time after calving and, in the modern milk production cycle, this variation is stretched over a long period, typically around 300 days, which can be divided into different stages of lactation.

Immediately after parturition (birth), the secretion of the mammary gland is termed colostrum, reflecting the fact that it is sufficiently different from the milk produced thereafter to be not regarded as milk in the true sense. Colostrum contains very high levels of nutrients needed in the first days of life, and hence is rich in fat and proteins such as immunoglobulins. The colostrum phase lasts around 48–72 hours, after which the secretion assumes a composition more typical of milk (Madsen *et al.*, 2004), and the next few weeks may be termed early lactation, which segues into the mid-lactation stage, during which milk yield is maximal and milk processing characteristics are typically at their optimal.

The contents of both total protein and casein are very high in very early lactation, and then rapidly decrease to reach their lowest level at 5–10 weeks of lactation, after which both increase gradually throughout the rest of lactation. The proportion of α_s- and κ-casein to total casein has been found to decrease with lactation, and the proportion of β-casein to total casein to increase with lactation, with the proportion of γ-caseins, degradation products of β-casein, being lowest in mid-lactation (Ostersen *et al.*, 1997). The cheese-making properties of milk are optimal in mid-lactation because of both the low level of

γ-casein and also the fact that the ratio of casein to total protein (the casein number) is highest in mid-lactation (Ng-Kwai-Hang *et al.*, 1982).

The last month or so of milk production may be termed late lactation. In some production environments, in which cows calve annually around the same time, late lactation coincides with advanced pregnancy, and the yield of milk decreases dramatically as the cow diverts energy and resources to the growing calf *in utero*. Eventually the yield becomes so low, and the composition and processing characteristics so sub-optimal, that milking is stopped ('drying off'). During late lactation, a number of progressive compositional changes occur, such as increasing pH, changing salts balance, increasing activity of proteolytic and lipolytic enzymes, and reduced level of casein as a proportion of total protein, all of which negatively impact on the manufacture of dairy products. In recent years, it has become accepted that there is a strong relationship between the quality of diet in late lactation and the quality of milk and dairy products, and that improving the quality of diet in late lactation may mitigate against some of the undesirable changes otherwise encountered (Kefford *et al.*, 1995).

1.5.2   Seasonality

Seasonal variations in milk composition are confounded with both lactation stage and feeding. In many countries, calving of cows occurs on a year-round basis, so that a mixture of milk of different stages of lactation is always available, diluting out the negative impact of late-lactation milk. In a small number of countries, however, particularly in Ireland and New Zealand, calving is highly synchronised, and most calves are born in the respective spring season, so that there is a pronounced seasonal pattern, with a lactational pattern dominating milk quality over the year and the availability of milk during winter months being very low. Auldist *et al.* (1998) reported the effects of seasonal and lactational influences on bovine milk composition in New Zealand. In Ireland, this has resulted in a dependency on large-scale manufacture of long shelf-life products (e.g., Cheddar cheese, milk powder, butter), with milk factories remaining closed for several months over the winter as the reduced volume of available milk is principally processed into liquid (drinking) milk. A reduced proportion of casein to total protein and poorer coagulation properties have been observed during late summer at grazing (Hermansen *et al.*, 1994). It is, furthermore, a well-known phenomenon that milk composition changes when the cows begin to graze on pasture, e.g. in Europe in springtime, where the dairies are often required to change recipes for cheese-making, especially at the beginning. The causes underpinning these changes in the milk composition are not fully understood, and are the subject of much current research.

1.5.3   Diet

As many milk constituents are produced from precursors obtained through the animals' diet, it is perhaps not surprising that the nature of the diet and nutritional status of the animal have a significant influence on the composition

of milk. Differences in milk composition may arise, for example, when animals are grazing on grass (see above) or when grass is not available and silage or concentrates are used.

The fatty acid composition of milk is partly dependent on the composition of the feed that the cow receives, as reviewed by Walker *et al.* (2004). Furthermore, ruminants can synthesise fatty acids via *de novo* synthesis. The *de novo* synthesis uses precursors for fatty acid synthesis resulting from the microbial fermentation of carbohydrates in the rumen, and consists primarily of acetic acid and butyric acid. Microbial fermentation, and thereby *de novo* synthesis, will be favoured by a diet with adequate roughage, while a diet including more concentrate will lower the production of precursors for *de novo* synthesis. The short-chain fatty acids, C4–C14, are all synthesised *de novo* by the cow, while palmitic acid (C16) is only partly formed by the cow, as it can also be derived from the feed. The long-chain fatty acids, such as stearic (C18), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids, are entirely derived from the feed or mobilised from the adipose tissue of the cow. Furthermore, ruminants have the ability to hydrogenate lipids in the rumen, and thereby saturate the unsaturated fatty acids derived from the feed. Hydrogenation is often not completed, and intermediates such as mono *trans* fatty acids and conjugated linoleic acid are formed. Finally, the cow has the ability to dehydrogenate saturated fatty acids in the udder by the enzyme d9-desaturase, and can, for example, desaturate stearic acid to oleic acid.

Accordingly, the fatty acid composition of the feed will affect the composition of the milk, and it has been found that the composition of roughage has an impact on the fatty acid composition and the oxidative stability of the milk (Havemose *et al.*, 2004). Grass–clover silage contains high concentrations of α-linolenic acid, which partly ends up in the milk, while maize silage contains a high amount of linoleic acid, consequently giving milk with high concentrations of linoleic acid. Use of oil-containing concentrates, such as linseed or soy, also affects the concentration of unsaturated fatty acids in the milk, thereby increasing the risk of oxidation (Timmons *et al.*, 2001).

In many countries the profile of fatty acids in milk fat is quite different in summer and winter as a result of changes in diet, and this necessitates changes in the procedure for manufacture of butter, to ensure that the hardness, spreadability and stability towards oxidation of the final product are optimised throughout the year. By grass feeding, the fatty acid profile is changed in the milk, resulting, for example, in a higher content of the beneficial fatty acid C18:3 (omega-3 fatty acid) which, however, is more prone to oxidation compared with saturated fat. Furthermore, feeding clover–grass or clover–grass silage will result in an increased level of vitamin E, an antioxidant (Havemose *et al.*, 2004).

The protein content and composition of milk are less influenced by diet compared with milk fat. A positive correlation between energy level in the feed and protein content in the milk has been reported (Spörndly, 1989). Feeding high levels of concentrate has been reported to increase the casein in relation to total

protein, and to result in a lower level of γ-caseins, which indicated decreased proteolysis (DePeters and Cant, 1992). The effect of diet on the protein and nitrogen fraction of bovine milk was reviewed by DePeters and Cant (1992), and the effects of nutritional factors on both the fat and protein fraction of milk was reviewed by Jenkins and McGuire (2006).

The link between diet and milk composition has led in recent years to interest in producing 'tailored' milks, whereby levels of desirable constituents (e.g., omega-3 fatty acids) may be increased, or those of less desirable constituents (e.g., saturated fatty acids) decreased by feeding strategies. There has also been extensive research on the impact of diet and nutrition on the yield and concentration of protein and fat, and on total milk yield.

1.5.4   Genetic factors

The hereditability of properties of milk (e.g., milk yield, yield or composition of fat and protein) has been studied in some detail, and is the subject of considerable current research. For example, a gene which not only has impact on the total fat content in milk, but also has profound effects on milk composition, is the DGAT 1 gene, which encodes for an enzyme participating in the synthesis of triglycerides in the udder. Substantial genetic variation in milk-fat composition has been shown to exist, and this variation was associated with different DGAT 1 variants (Schennink *et al.*, 2007). Heritabilities were found to be high for short- and medium-chain fatty acids (C4:0–C16:0) and moderate for long-chain fatty acids (saturated and unsaturated C18). Breeding strategies have resulted in an up-regulation of the DGAT1 A allele, which contributes to a higher total fat content in milk, in addition to a generally elevated level of saturated fat (Zock *et al.*, 1994).

Many studies have focused on the effects of individual milk protein genotypes on protein content and composition and in relation to some processing criteria. In addition, specific constituents may be influenced by genetic alleles, with several genetic variants of most milk proteins being commonly found, for example, that differ by changes in one or more amino acids in the protein sequence. There have been a number of studies of the relationship between genetic variants and milk processing characteristics; fewer studies have included the effects of combined genotypes, i.e., cow haplotypes, which are of course more complicated to conduct.

Those known genotypes with the most pronounced known effects on milk protein composition and processing characteristics include the genetic variants of κ-casein and β-lactoglobulin. Compared with the A variant, the B variant of κ-casein is correlated with a higher content of total protein, caseins, κ-casein and Ca^{2+}; of the κ-casein variants, the BB variant has been shown to give the shortest coagulation time during cheese-making (van den Berg *et al.*, 1992). The B variant of β-lactoglobulin has been correlated with a higher casein content relative to total protein, and accordingly with a higher cheese yield (van den Berg *et al.*, 1992). This is primarily due to the β-lactoglobulin content, but not the casein

content, being higher in AA milks. For β-casein, milks that contain the B variant, and especially BB-type milks, have a shorter rennet coagulation time and better coagulum strength than the A^2A^2 variants (Jakob and Puhan, 1992).

As the gene loci for the caseins are closely linked at chromosome VI, the alleles of the different caseins are in linkage disequilibrium. This means that the casein alleles are not independently distributed, i.e., there are significant interactions between the casein loci. This was also found by Mayer *et al.* (1997), who studied effects of the combined genotypes of β-casein (A^2A^2, A^2B), κ-casein (AA, AB and BB) and β-lactoglobulin (AA, BB), i.e., the significance of different haplotypes in relation to composition and cheese-making properties. The study confirmed that the casein number was higher for β-lactoglobulin BB milk than for AA milk, and that the concentration of κ-casein increased from AA to BB milk. Furthermore, recovery for milk solids was better for β-casein A^2B than for A^2A^2. Therefore, generally speaking, the B variants of β-casein, κ-casein and β-lactoglobulin are favourable for coagulation and cheese-making properties of milk.

The complete bovine genome is now known (Sonstegard and van Tassell, 2004). New biotechnological methods for genotyping have been developed by which large numbers (more than 50,000) of gene markers can be genotyped simultaneously. This opens possibilities of new insights in the future into those parts of the hereditary material that are decisive for variation in milk composition and technological properties across populations.

1.5.5   Breed

Different breeds of cows are known to differ in the yield and composition of the milk they produce. This is due both to differences in the capacity for synthesis between different breeds, but also to differences in allele frequencies of various milk proteins between breeds. Among Danish breeds, studies have shown that not only the fat and casein content, but also the casein number, were higher for Jersey cows than for the Danish black and white (SDM) Holstein Friesians, being 4.08 and 3.37 for the protein content and 79.5 and 77.5 for the casein number, respectively, in the herd studied (L.B. Larsen, unpublished results). For this reason, and due to the frequency of κ-casein B allele being high in Jersey milk, milk from Jerseys is considered to be superior for cheese-making compared with, e.g., Holstein-Friesians, which, on the other hand, have a higher milk yield. Auldist *et al.* (2004) compared the cheese-making properties of milk from Jersey and Friesian cows, and related the faster rennet coagulation and firmer curd for the former milk type to its higher concentration of solids. Also the average fat globule size varies among breeds, being, e.g., ~4 μm for Holstein-Friesians and ~5.5 μm for Jerseys.

Another Scandinavian study revealed that, in a herd of Swedish Red and White cows (SRB) and Swedish Holstein cows (SLB), the concentrations of total protein, total casein, β-casein and κ-casein were significantly higher in SRB milk compared with SLB milk (Wedholm *et al.*, 2006).

1.5.6   Mastitis and somatic cell count

One of the most dramatic factors affecting the composition and quality of milk is mastitis, which is defined as inflammation of the mammary gland, generally resulting from bacterial invasion of the gland and the resulting response of the immune system in fighting this invasion. The primary defensive mechanism involves a massive influx of somatic cells into milk; numbers of these cells, which are present at low levels in milk from healthy animals, can increase within hours by a factor of 10–20, with somatic cell counts (SCC) of over 1,000,000 cells/ml being common in the throes of an infection, with numbers decreasing again if and when the infection is controlled. The function of the cells ranges from direct phagocytosis and killing of bacteria (polymorphonuclear leucocytes and macrophages) to immune functions (lymphocytes).

During mastitis, the secretory function of the udder is significantly compromised by the ongoing response; and unsurprisingly, the composition of milk changes dramatically (see Table 1.2); for example, the influx of blood constituents (e.g., enzymes, immunoglobulins) increases significantly as the barrier separating the two fluids becomes more permeable to facilitate the entry of somatic cells and shifts in the balance of mineral salts, leading to a significant decrease in lactose concentration to maintain osmotic pressure. The influx of blood proteins and the synthesis of defence proteins by the udder lead to an increase in the levels and change in the composition of proteins, especially of the whey and the MFGM proteins in mastitic milk (Hogarth *et al.*, 2004; Smolenski *et al.*, 2007).

The enzymes arising from cells and blood hydrolyse the casein, and in severe cases up to 50% of the casein can be hydrolysed, leading to the generation of a complex pattern of degradation products, i.e. casein peptides and larger fragments (Larsen *et al.*, 2004; Mehrzad *et al.*, 2005; Wedholm *et al.*, 2008). This proteolysis reduces the shelf-life of pasteurised milk, impairs the processing properties of the milk, such as rennet gelation, and reduces cheese yield (Auldist and Hubble, 1998; Ma *et al.*, 2000). Furthermore, during mastitis, enzymes originating from the microbial pathogen may also be present in the milk and can affect the milk quality, e.g. by proteolysis. The fact that proteolysis is increased in mastitic milk is well established, but the actual level of degraded caseins is not easy to predict. The proteolysis occurring is influenced by a complex set of factors relating to both the type and stage of infection, lactation physiology and management, in addition to large individual variances in the response to infection between animals.

In cases of severe mastitis with obvious symptoms (clinical mastitis) the milk should not be used for further processing, and the animal may be treated with antibiotics, which also precludes its use for human consumption. However, at any time a significant proportion of animals in a herd may be infected with subclinical mastitis, symptoms of which are less obvious, and which is associated with much smaller increases in SCC; hence, such milk may enter the bulk collection in the farm and ultimately be used for processing. This has led to the question of what is the recommended upper limit for SCC of milk to be processed, which in the EU is currently at 400,000 cells/ml.

Table 1.2   Summary of changes in milk components at mastitis

Component	Effect	Component	Effect
Fat	?	α_s-casein	↓
Free fatty acids	↑	β-casein	↓
Lactose	↓	κ-casein	?
Total protein	?	γ-casein	↑
Total casein	↓	α-lactalbumin	↓
Whey proteins	↑	β-lactoglobulin	↓
Casein:total protein	↓	Serum albumin	↑
Non-casein N	↑	Immunoglobulin G	↑
Peptides	↑	Lactoferrin	↑
Na	↑	Transferrin	↑
K	↓	Plasmin	↑
Total Ca	↓	Somatic cell proteases	↑
Cl	↑	Acute phase proteins	↑

Source: Modified from Auldist and Hubble (1998).

The infection or elevated SCC is normally not observed in all glands of an infected cow, and this leads to variations in the composition of the milk proteins at gland level (Ureh *et al.*, 1999; Lindmark-Månsson *et al.*, 2006; Larsen *et al.*, 2004). Even though new milking systems, i.e. automatic milking systems, may permit the sorting of milk at gland level, this cannot be recommended as it has been shown that the quality of the milk from uninfected glands next to infected glands can be severely affected by proteolysis, probably by plasmin (Larsen *et al.*, 2004). The changes in milk composition and the effects on the processing characteristics in mastitis were reviewed by Auldist and Hubble (1998) and are summarised in Table 1.2.

1.6   Conclusions

Milk is a highly complex raw material for processing, in terms of both the properties of its constituents and their susceptibility to change on processing. In addition, the levels of constituents are not static but rather highly dynamic, and susceptible to change due to a wide range of factors. Thus, production of high-quality dairy products requires consideration of factors right from the cow (or other mammal) through to the factory.

1.7   References

AULDIST MJ, HUBBLE IB (1998), 'Effects of mastitis on raw milk and dairy products', *Aust J Dairy Technol*, 53, 28–36.

AULDIST MJ, WALSH BJ, THOMSON NA (1998), 'Seasonal and lactational influences on bovine milk composition in New Zealand', *J Dairy Res*, 65, 401–411.

AULDIST MJ, JOHNSTON KA, WHITE NJ, FITZSIMONS WP, BOLAND MJ (2004), 'A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Fresian and Jersey dairy cows', *J Dairy Res*, 71, 51–57.

BARREFORS P, GRANELLI K, APPELQVIST A-L, BJOERCK L (1995), 'Chemical characterisation of raw milk samples with and without oxidative off-flavour', *J Dairy Sci*, 78, 2691–2699.

BASTIAN ED, BROWN RJ (1996), 'Plasmin in milk and dairy products: an update', *Int Dairy J*, 6, 435–457.

BAUMAN DE, MATHER IH, WALL RJ, LOCK AL (2006), 'Major advances associated with the biosynthesis of milk', *J Dairy Sci*, 89, 1235–1243.

BENFELDT C, LARSEN LB, RASMUSSEN JT, ANDREASEN PA, PETERSEN TE (1994), 'Isolation and characterisation of plasminogen and plasmin from bovine milk', *Int Dairy J*, 5, 577–592.

BISIG W, EBERHARD P, COLLUMB M, REHBERGER B (2007), 'Influence of processing on the fatty acid composition and the content of conjugated linoleic acid in organic and conventional dairy products – a review', *Lait*, 87, 1–19.

CAPUCO AV, AKERS RM (2002), 'Galactopoesis, effect of bST treatment', in Roginski H, Fuquay JW and Fox PF, *Encyclopedia of Dairy Sciences*, San Diego, Academic Press, 1458–1464.

CHILLIARD Y, GLASSER F, ENJALBERT F, FERLAY A, BOCQUIER F, SCHMIDELY PH (2007), 'Recent data on the effects of feeding factors on milk fatty acid composition in the cow, goat and ewe', *Rencontres Autour des Recherches sur les Ruminants*, 14, 321–328.

COLLOMB M, SCHMID A, SEIBER R, WESCHLER D, RYHAANEN EL (2006), 'Conjugated linoleic acids in milk fat: variation and physiological effects', *Int Dairy J*, 11, 1347–1361.

CONSIDINE T, HEALY A, KELLY AL, MCSWEENEY PLH (2004). 'Hydrolysis of bovine caseins by cathepsin B, a cysteine proteinase indigenous to milk', *Int Dairy J*, 14, 117–124.

DEETH HC (2006), 'Lipoprotein lipase and lipolysis in milk', *Int Dairy J*, 16, 555–562.

DE KRUIF CG, HOLT C (2003), 'Casein micelle structure, functions and interactions', in Fox PF and McSweeney PLH (eds), *Advanced Dairy Chemistry, Volume 1: Proteins*, 3rd edn, New York, Kluwer Academic/Plenum Publishers, 233–276.

DEPETERS EJ, CANT JP (1992), 'Nutritional factors influencing the nitrogen composition of bovine milk: A review', *J Dairy Sci*, 75, 2043–2070.

ENATTAH NS, JENSEN TGK, NIELSEN M, LEWINSKY R, KUOKKANEN M, RASINPERA H, EL-SHANTI H, SEO JK, ALIFRANGIS M, KHALIL IF, NATAH A, ALI A, NATAH S, COMAS D, MEHDI SQ, GROOP L, VESTERGAARD EM, IMTIAZ F, RASHED MS, MEYER B, TROELSEN J, PELTONEN L (2008), 'Independent introduction of two lactase-persistent alleles into human populations reflects different history of adaptation to milk culture', *Am J Hum Gen*, 82, 57–72.

ENRIGHT E, BLAND AP, NEEDS EC, KELLY AL (1999), 'Proteolysis and physicochemical changes in milk on storage as affected by UHT treatment, plasmin activity and KIO$_3$ addition', *Int. Dairy J*, 9, 581–591.

FARKYE NY, FOX PF (1992), 'Contribution of plasmin to Cheddar cheese ripening: Effect of added plasmin', *J Dairy Res*, 59, 209–216.

FONG BY, NORRIS CS, MACGIBBON AKH (2007), 'Protein and lipid composition of bovine milk-fat-globule membrane', *Int Dairy J*, 17, 275–288.

FONG BY, NORRIS CS, PALMANO KP (2008), 'Fractionation of bovine whey proteins and characterisation by proteomic techniques', *Int Dairy J*, 18, 23–43.

FORD JE, SCHRÖDER MJA, BLAND MA, BLEASE KS, SCOTT KJ (1986), 'Keeping quality of milk in relation to the copper content and temperature of pasteurisation', *J Dairy Res*, 53, 391–406.

FOX PF, KELLY AL (2004) 'The caseins', in Yada R (ed.), *Proteins in Food Processing*, Cambridge, Woodhead Publishing, 29–71.

FOX PF, KELLY AL (2006a), 'Indigenous enzymes in milk: overview and historical aspects – Part 1', *Int Dairy J*, 16, 500–516.

FOX PF, KELLY AL (2006b), 'Indigenous enzymes in milk: overview and historical aspects – Part 2', *Int Dairy J*, 16, 517–532.

HARRISON R (2006), 'Milk xanthine oxidase: properties and physiological roles', *Int Dairy J*, 16, 546–564.

HAVEMOSE MS, WEISBJERG MR, BREDIE WLP, NIELSEN JH (2004), 'Influence of different types of roughage on the oxidative stability of milk', *Int Dairy J*, 14, 563–570.

HAYES MG, HURLEY MJ, LARSEN LB, HEEGAARD CW, MAGBOUL AAA, OLIVEIRA JC, MCSWEENEY PLH, KELLY AL (2001), 'Thermal inactivation kinetics of bovine cathepsin D', *J Dairy Res*, 68, 267–276.

HERMANSEN JE, OSTERSEN S, AAES O (1994), 'Effects of the levels of N fertilizer, grass, and supplementary feeds on nitrogen composition and renneting properties of milk from cows at pasture', *J Dairy Res*, 61, 179–189.

HOGARTH CJ, FITZPATRICK JL, NOLAN AM, YOUNG FJ, PITT A, ECKERSAIL PD (2004), 'Differential composition of bovine whey: a comparison of whey from healthy animals and from those with clinical mastitis, *Proteomics*, 4, 2094–2100.

HOLLAND JW, DEETH HC, ALEWOOD PF (2004), 'Proteomic analysis of κ-casein micro-heterogeneity', *Proteomics*, 4, 743–752.

HORNE DS (2003), 'Caseins, molecular structure', in Fox PF and McSweeney PLH (eds), *Encyclopedia of Dairy Sciences*, Amsterdam, Academic Press, 1902–1909.

HUPPERTZ T, KELLY AL (2006), 'Physical chemistry of milk fat globules', in Fox PF and McSweeney PLH (eds), *Advanced Dairy Chemistry 2 (Lipids)*, 3rd edn, New York, Kluwer Academic/Plenum Publishers, 173–212.

HUPPERTZ T, KELLY AL, FOX PF (2008), 'Milk fat production', in Tamime AT (ed.), *Dairy Fat and Related Products*, London, Blackwell Academic (in press).

HURLEY MJ, LARSEN LB, KELLY AL, MCSWEENEY PLH (2000), 'Cathepsin D activity in quarg', *Int Dairy J*, 10, 453–458.

JAKOB E, PUHAN Z (1992), 'Technological properties of milk as influenced by genetic polymorphism of milk proteins – a review', *Int Dairy J*, 2, 157–178.

JENKINS TC, MCGUIRE MA (2006), 'Major advances in nutrition: Impact on milk composition', *J Dairy Sci*, 89, 1302–1310.

JENSEN RG (2002), 'Invited review – The composition of bovine milk lipids: January 1995 to December 2000', *J Dairy Sci*, 85, 295–350.

KEFFORD B, CHRISTIAN MP, SUTHERLAND BJ, MAYES JJ, GRAINER C (1995), 'Seasonal influences on Cheddar cheese manufacture – influence of diet quality and stage of lactation', *J Dairy Res*, 62, 529–537.

KELLY AL, FOX PF (2006), 'Indigenous enzymes in milk: a synopsis of future research requirements', *Int Dairy J*, 16, 707–715.

KELLY AL, O'FLAHERTY F, FOX PF (2006), 'Indigenous proteolytic enzymes in milk: an overview of the present state of knowledge', *Int Dairy J*, 16, 563–572.

KOHLMANN KL, NIELSEN SS, LADISCH MR (1991), 'Effect of a low concentration of added plasmin on ultra-high temperature processed milk', *J Dairy Sci*, 74, 1151–1156.

LACY-HULBERT SJ, WOOLFORD MW, NICHOLAS GD, PROSSER CK, STELWAGEN K (1999), 'Effect

of milking frequency and pasture intake on milk yield and composition of late lactation cows', *J Dairy Sci*, 82, 1232–1239.

LARSEN LB, PETERSEN TE (1995), 'Identification of five molecular forms of cathepsin D in bovine milk', in Takahashi K, *Aspartic Proteinases: Structure, Function, Biology, and Biomedical Implications*, Vol. 362, New York, Plenum Press, 279–283.

LARSEN LB, BOISEN A, PETERSEN TE (1993), 'Procathepsin D cannot autoactivate to cathepsin D at acid pH', *FEBS Lett*, 319(1–2), 54–58.

LARSEN LB, BENFELDT C, RASMUSSEN LK, PETERSEN TE (1996), 'Bovine milk procathepsin D and cathepsin D: coagulation and milk protein degradation', *J Dairy Res*, 63, 119–130.

LARSEN LB, WIUM H, BENFELDT C, HEEGAARD CW, ARDÖ Y, QVIST KB, PETERSEN TE (2000), 'Bovine milk procathepsin D: Presence and activity in heated milk and in extracts of rennet-free UF-feta cheese', *Int Dairy J*, 10, 67–73.

LARSEN LB, RASMUSSEN MD, BJERRING M, NIELSEN JH (2004), 'Proteases and protein degradation in milk from cows infected with *Streptococcus uberis*', *Int Dairy J*, 14, 899–907.

LARSEN LB, MCSWEENEY PLH, HAYES MG, ANDERSEN JB, INGVARTSEN KL, KELLY AL (2006), 'Variation in activity and heterogeneity of bovine milk proteases with stage of lactation and somatic cell count', *Int Dairy J*, 16, 1–8.

LINDMARK-MÅNSSON H, FONDÉN R, PETTERSSON H-E (2003), 'Composition of Swedish dairy milk', *Int Dairy J*, 13, 409–425.

LINDMARK-MÅNSSON H, BRÄNNING C, ALDÉN G, PAULSSON M (2006), 'Relationship between somatic cell count, individual leucocyte populations and milk components in bovine udder quarter milk', *Int Dairy J*, 16, 717–727.

MA Y, RYAN C, BARBANO DM, GALTON DM, RUDAN MA, BOOR KJ (2000), 'Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk', *J Dairy Sci*, 83, 264–274.

MADSEN BD, RASMUSSEN MD, NIELSEN MO, WIKING L, LARSEN LB (2004), 'Physical properties of mammary secretions in relation to chemical changes during transition from colostrum to milk', *J Dairy Res*, 71, 263–272.

MAGBOUL AAA, LARSEN LB, MCSWEENEY PLH, KELLY AL (2001), 'Cysteine protease activity in bovine milk', *Int Dairy J*, 11, 865–872.

MARA O, ROUPIE C, DUFFY A, KELLY AL (1998), 'Influence of plasmin hydrolysis on the curd-forming properties of milk', *Int. Dairy J*, 8, 807–812

MATHER IH, KEENAN TW (1998), 'Origin and secretion of milk lipids', *J Mammary Gland Biol Neopl*, 3, 259–273.

MAYER HK, ORTNER M, TSCHAGER E, GINZINGER W (1997), 'Composite milk protein phenotypes in relation to composition and cheesemaking properties of milk', *Int Dairy J*, 7, 305–310.

MCINTOSH G, ROUPAS P, ROYLE P (2006), 'Cheese, omega-3 fatty acids, conjugated linoleic acid and human health', *Aust J Dairy Technol*, 61, 142–146.

MCSWEENEY PLH, FOX PF, OLSON NF (1995), 'Proteolysis of bovine caseins by cathepsin D: Preliminary observations and comparison with chymosin', *Int Dairy J*, 5, 321–336.

MEHRZAD J, DESROSIERS C, LAUZON K, ROBITAILLE G, ZHAO X, LACASSE P (2005), 'Proteases involved in mammary tissue damage during endotoxin-induced mastitis in dairy cows', *J Dairy Sci*, 88, 211–222.

NG-KWAI-HANG KF, HAYES JF, MOXLEY JE, MONARDES HG (1982), 'Environmental influences on protein content and composition of bovine milk', *J Dairy Sci*, 65(10), 1993–1998.

OSTDAL H, ANDERSEN HJ, NIELSEN JH (2000), 'Antioxidative activity of urate in bovine milk', *J Agric Food Chem*, 48, 5588–5592.

OSTERSEN S, FOLDAGER J, HERMANSEN JE (1997), 'Effects of stage of lactation, milk protein genotype and body condition at calving on protein composition and renneting properties of bovine milk', *J Dairy Res*, 64, 207–219.

RASMUSSEN LK, JOHNSEN LB, TSIORA A, SØRENSEN ES, THOMSEN JK, NIELSEN NC, JAKOBSEN HJ, PETERSEN TE (1999), 'Disulphide-linked caseins and casein micelles', *Int Dairy J*, 9, 215–218.

REINHARDT TA, LIPPOLIS JD (2006), 'Bovine milk fat globule membrane', *J Dairy Res*, 73, 406–416.

RICHARDSON BC (1983), 'The proteinases of bovine milk and the effect of pasteurisation on their activity', *NZ J Dairy Sci Technol*, 18, 245–252.

RICHARDSON BC, PEARCE KN (1981), 'The determination of plasmin in dairy products', *NZ J Dairy Sci Technol*, 16, 209–220.

SCHENNINK A, STOOP WM, VISKER HPWM, HECK JML, BOVENHUIS H, VAN DER POEL JJ, VAN VALENBERG HJF, VAN ARENDONK JAM (2007), 'DGAT 1 underlies large genetic variation in milk fat composition of dairy cows', *Anim Genet*, 38, 467–473.

SILANIKOVE N, SHAMAY A, SHINDER D, MORAN A (2000), 'Stress downregulates milk yield in cows by plasmin induced β-casein product that blocks K$^+$ channels on the apical membranes', *Life Sciences*, 67, 2201–2212.

SILANIKOVE N, MERIN U, LEITNER G (2006), 'Physiological role of indigenous milk enzymes: an overview of an evolving picture', *Int Dairy J*, 16, 533–545.

SMOLENSKI G, HAINES S, KWAN F Y-S, BOND J, FARR V, DAVIS SR, STELWAGEN K, WHEEKER TT (2007), 'Characterisation of host defence proteins in milk using a proteomic approach', *J Proteome Res*, 6, 207–215.

SONSTEGARD TS, VAN TASSELL CP (2004), 'Bovine genomics update: making a cow jump over the moon', *Genet Res Camb*, 84, 3–9.

SPÖRNDLY E (1989), 'Effects of diet on milk composition and yield of dairy cows with special emphasis on milk protein content', *Swedish J Agric Res*, 19, 99–106.

SWAISGOOD HE (1992), 'Chemistry of the caseins', in Fox PF, *Advanced Dairy Chemistry, Proteins, Vol. 1*, London and New York, Elsevier Applied Science, 63–110.

TIMMONS JS, WEISS WP, PALMQUIST DL, HARPER WJ (2001), 'Relationships among dietary roasted soybeans, milk components, and spontaneous oxidized flavour of milk', *J Dairy Sci*, 84, 2440–2449.

UREH E, PUHAN Z, SCHÄLLIBAUM M (1999), 'Changes in milk protein fractions as affected by subclinical mastitis', *J Dairy Sci*, 82, 2402–2411.

VAN DEN BERG G, ESCHER JTM, DE KONING PJ, BOVENHUIS H (1992), 'Genetic polymorphism of κ-casein and β-lactoglobulin in relation to milk composition and processing properties', *Neth Milk Dairy J*, 46, 145–168.

WALKER GP, DUNSHEA FR, DOYLE PT (2004), 'Effects of nutrition and management on the production and composition of milk fat and protein: a review', *Aust J Agric Res*, 55, 1009–1028.

WEDHOLM A, HALLÉN E, LARSEN LB, LINDMARK-MÅNSSON H, KARLSSON AH, ALLMERE T (2006), 'Comparison of milk protein composition in a Swedish and a Danish dairy herd using reversed phase HPLC', *Acta Agriculturae Scand Section A*, 56, 8–15.

WEDHOLM A, MØLLER HS, LINDMARK-MÅNSSON H, RASMUSSEN MD, ANDRÉN A, LARSEN LB (2008), 'Identification of peptides in milk as a result of proteolysis at different levels of somatic cell counts using LC MALDI spotting and MS/MS detection', *J Dairy Res*, 75, 76–83.

WIKING L, BJÖRCK L, NIELSEN JH (2003), 'Influence of feed composition on stability of fat globules during pumping of raw milk', *Int Dairy J*, 13, 797–803.

WIKING L, BERTRAM HC, BJÖRCK L, NIELSEN JH (2005), 'Evaluation of cooling strategies for pumping of milk – Impact of fatty acid composition on free fatty acid levels', *J Dairy Res*, 72, 476–481.

ZOCK PL, DEVRIES JHM, KATAN MB (1994), 'Impact of myristic acid versus palmitic acid on serum-lipid and lipoprotein levels in healthy women and men', *Arterosclerosis and Thrombosis*, 14(4), 567–575.

2

The microbiological safety of raw milk

M. W. Griffiths, University of Guelph, Canada

Abstract: This chapter reviews the safety and benefits of raw milk. There are many sources of contamination of milk, including the cow itself, the environment, water and milking equipment. The varied routes for introduction of pathogens into milk preclude the production of milk that can be guaranteed to be safe for consumption. This is borne out by the many studies that show that pathogens can be isolated from a low but significant percentage of raw milks produced under hygienic conditions. The most effective way to reduce the risks of foodborne illness from the consumption of milk is by pasteurization.

Because it is practically impossible to eliminate pathogens from raw milk, outbreaks of foodborne illness continue to be attributed to raw milk. The main group affected are young children who have no choice in what they consume. There is also the possibility that people who drink raw milk can become ill or become asymptomatic carriers of disease and transmit illness by person-to-person contact to non-consumers of raw milk. Farmers themselves can acquire infections through the consumption of raw milk or be asymptomatic carriers. In a significant percentage of cases, there can be severe consequences resulting from the contraction of foodborne illness, including long-term neurological damage, arthritis and kidney failure.

It has been suggested that raw milk contains agents that inactivate pathogens. However, many of these agents survive pasteurization and are present in pasteurized milk. It has also been proposed that raw milks contain bacteria that are beneficial to human health, but they are unlikely to be present at levels that produce these effects.

Key words: milk-borne pathogens, antimicrobial activity, raw milk.

2.1   Microbial contamination of milk

Milk contains proteins, carbohydrates, lipids, vitamins and minerals and its primary role is to provide nourishment to the neonates of the mammalian species from which it was derived. However, milk from a variety of animals has become an important and valuable part of the human diet. These same components that make it nutritious for humans also provide an ideal growth medium for many microorganisms, including potential pathogens.

Although milk production practices differ greatly throughout the world, in most developed countries milk is collected by machine milking and transferred to refrigerated bulk storage tanks where it is held prior to transportation. These handling methods have resulted in a dramatic change in the microflora of raw milk brought about by selection and adaptation. The microorganisms present in milk can be introduced by a variety of routes.

2.1.1   Contamination from the udder

Contamination from udder infection

In healthy cows free from infection, milk emerging from the udder is essentially sterile, but it may contain commensal bacteria associated with the udder. A commensal organism derives food or other benefits from another organism without affecting it. These are usually members of the genera *Micrococcus* and *Streptococcus* as well as coryneform bacteria (members of a particular family of bacteria named Corynebacteriaceae) and occasionally coliforms (a group of bacteria commonly found in the gastrointestinal tract of animals that ferment the sugar lactose) (160). Significant numbers of organisms are found in milk taken in a manner that prevents microbial contamination (i.e. aseptically) from the udders of apparently healthy cows. Limond and Griffiths (84) obtained counts of about 100 cfu/ml in aseptically drawn milk, although no attempt was made to identify these isolates.

However, udder infections are common and in a nationwide study on 106 Canadian dairy farms the incidence rate of clinical mastitis in Canada was found to average 23 cases per 100 cow-years (or lactation periods) (106). Mastitis is defined as an inflammation of the mammary gland or udder; it can be subclinical in which there are no visible signs of infection, clinical in which there are signs of infection, or chronic when the symptoms persist over a long period of time. The most common agents of mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Str. uberis* and *Escherichia coli*. In the Canadian study, the distribution of pathogens differed over regions, with categories of bulk milk somatic cell count, and barn type. The somatic cell count (SCC) is one indicator of the quality of milk. Herds in tie-stall barns had greater incidence rates of mastitis caused by *Staphylococcus aureus*, coagulase-negative staphylococci and *Streptococcus uberis*, whereas herds in free-stalls had greater incidence rates of mastitis caused by *Escherichia coli* and *Klebsiella* spp.

Interestingly, no association was found between bulk milk somatic cell count and overall incidence rate of clinical mastitis (IRCM), but *E. coli* and cases of

clinical mastitis where no causative agent could be detected were highest and *Staph. aureus* IRCM was lowest in herds with low (<150,000 cells/ml) and medium (151,000–250,000 cells/ml) somatic cell counts. This suggests that somatic cell count cannot be relied upon to indicate the absence of pathogens in milk.

As well as producing visible clinical infection, less acute, subclinical states are often encountered and can only be diagnosed by laboratory examination of the milk for characteristic changes, such as elevated somatic cell counts and increases in concentrations of serum albumin and immunoglobulins in the milk. Thus, milk from cows with subclinical mastitis often finds its way into the milk supply. The organisms enter the udder by way of the duct at the teat tip and some, such as *Staph. aureus*, can colonize the duct. It is thought that machine milking plays a part in the propulsion of the organisms into the teat duct but this is by no means the only route of contamination (12). From the duct the organisms can enter the milk and can contribute significantly to the numbers present in bulk tank samples. Bramley *et al.* (13) isolated *Staph. aureus* or mastitis-producing streptococci from 86% of milk samples analyzed, but bacterial numbers were below 1×10^4 cfu/ml in 90% of these milks. However, in herds with a high incidence of *Str. uberis* infections, counts in the bulk tank milk reached 1×10^5 cfu/ml in a small number of cases. To put this into context, Ontario farmers incur a financial penalty when counts in their milk exceed 4.9×10^4 cfu/ml. The results from one study have suggested subclinical mastitis is a greater problem in organic than in conventional production systems, but the differences were not marked (124).

Whereas the organisms that cause mastitis do not generally grow in refrigerated milk, they are able to survive under these conditions and may be a concern from a public health aspect. For example, it has been demonstrated that staphylococcal enterotoxins and toxic shock syndrome toxin-1 can be preformed in the udder and secreted into milk in cows and goats suffering from *Staph. aureus* mastitis (105, 152). Ingestion of the toxin in the milk may result in illness. Modern dairy husbandry practices, such as teat dipping and dry cow therapy with antibiotics, can successfully reduce the incidence of mastitis. Dry cow therapy involves intramammary injection of antibiotics following the last milking of the lactation period to rid the mammary gland of potential pathogens in readiness for the next lactation. Hillerton *et al.* (63) showed that application of a mastitis control plan reduced the incidence of cows infected with mastitis caused by coagulase-positive staphylococci from 21.9% to 12.0% in a five year period. However, the total incidence of clinical mastitis did not change significantly because environmental organisms were responsible for 65% of all clinical cases. This indicates that udder infections may not be entirely eliminated by adopting good husbandry practices.

Apart from mastitis-causing organisms, other bacteria that are pathogenic to humans may infect the udder; these include *Mycobacterium bovis*, which can cause tuberculosis in humans (54), *Brucella abortus* (the causative agent of brucellosis or undulant fever, which is a highly contagious disease caused by

ingestion of raw milk or meat from infected animals, or close contact with their secretions, such as milk), *Listeria monocytogenes*, *Coxiella burnetii* and *Salmonella* spp. (150). For example, *C. burnetii* does not cause clinical disease in cattle, but it gives rise to Q fever in humans. It has been detected, using a polymerase chain reaction (PCR)-based assay, in 94% of pooled milk collected on farms in the US (77). Recent attention has focused on *Mycobacterium paratuberculosis*, the causal agent of Johne's disease, a chronic, progressive gastroenteritis of ruminants which has also been linked to Crohn's disease in humans (52, 53). It has been estimated that at least 68% of all US dairy herds are infected with this organism (77).

Contamination from the external surface of the udder
The external surface of the udder is also a prime source of microbial contamination of milk. Bedding materials, mud, feces, soil and other matter all readily stick to skin and are a rich source of microorganisms. Even after washing with water, the microbial count on teat surfaces can be high (147) and the count in milk from washed udders may only be about 1 log cycle lower than from those that were unwashed (72). Similar low-level reductions in total microbial count and coliform counts on both the udder surface and in milk were observed even after the use of disinfectants to treat teats (44, 46). However, the importance of proper washing and drying of the udder before milking for the elimination of *Listeria* spp. has been demonstrated (67). In a study on the risk factors associated with contamination of raw milk by *Listeria monocytogenes* on dairy farms, Sanaa *et al.* (127) showed that poor cleanliness of cows, inadequate lighting of milking parlors and barns (which may be an indication of neglect of milking hygiene) and incorrect disinfection of towels used to dry the udder significantly increased the likelihood of contamination.

To determine the relevance of the exterior of the udder as a source of bulk tank milk contamination, Vissers *et al.* (156) calculated the amount of dirt transmitted to milk via the exterior of teats. They chose 11 Dutch farms at random and used spores of bacteria as markers for transmitted dirt. The amount of dirt transmitted to milk varied among farms from 3 to 300 mg/L, with an average of 59 mg/L. Using this figure it is possible to carry out a rough risk assessment. For example, if it is assumed that a serving of raw milk consists of 250 ml (one cup) and that the infectious dose of *E. coli* O157:H7 is 10 cells for a susceptible individual such as a child, then a level of contamination equivalent to one cell of *E. coli* per mg (1000 cfu/g) of dirt would be sufficient to achieve a potentially hazardous dose. Even at the lowest rate of transmission (3 mg/L), an infectious dose would be achieved with counts of about 13 cfu/mg (13,000 cfu/g). This is within the lower limit of the range of counts of *E. coli* O157:H7 shed in feces by 'super-shedders' (19).

It has been suggested that bedding affords the greatest contribution to external udder contamination (133). There was a reduction in bacterial levels on teats when cows were on pasture and this was reflected in lower bacterial counts in milk during this period. The bacterial count of all types of bedding was about

5×10^9 cfu/g whereas that on pasture was approximately 8×10^7 cfu/g (93). A recent study conducted at AgResearch MIRINZ suggests that off-pasture management of dairy cows leads to increased carriage of foodborne pathogens (162). For example, the percent prevalence of *E. coli* O157:H7 increased from about 48% for pasture-raised cows to about 95% for cows raised using feed pad/ pasture or herd home/pasture systems. It should be noted, however, that this was a limited study involving only three farms.

The dominant microflora on the teats of cows housed in barns were micrococci (148) but it has also been estimated that 90% of the spores found in raw milk come from this source (93). The principal source of psychrotrophic spore-formers (mainly *Bacillus* spp.) in milk appears to be contamination of the teat by the upper layer of soil in pasture land and by feces (93, 146) but this obviously does not hold for cows that are zero-grazed. There is also a distinct seasonal effect on the incidence of psychrotrophic spore-formers in milk, with the highest levels being observed in late summer and early autumn (93, 118, 142). McKinnon and Pettipher (93) determined that the mean total spore count in farm bulk tank milks during the winter was 2.2×10^2 cfu/ml, whereas the corresponding value in the summer was 10 cfu/ml. *Clostridium* spores can be introduced into milk from feedstuff, especially silage, and bedding (33, 62). Silage is also an important source of contamination by *Listeria* spp., including *L. monocytogenes*, and other potential human pathogens such as *Yersinia enterocolitica* and *Aeromonas hydrophila* (50, 127).

2.1.2   Fecal shedding

Although bedding can be a significant source of udder contamination, arguably fecal contamination plays a more significant role. Several potential human pathogens are naturally present in the intestinal tract of cattle and these animals do not show signs of infection. For example, *Campylobacter jejuni* is one of the most common causes of human gastroenteritis in the world. Illness caused by *Campylobacter* species can be severely debilitating but is rarely life-threatening. *Campylobacter jejuni* has very specific growth requirements that hamper its ability to grow in feed and in the environment. However, these growth requirements make it ideally suited to life in the intestinal tract of animals and birds and the feces of asymptomatic 'carrier' animals are considered to be the major reservoir for this pathogen (107). A similar route of dissemination has been proposed for *Listeria monocytogenes* (104), whereby the organism is shed into the environment by cattle following ingestion of contaminated feed. Perhaps the most important pathogens shed into the feces by asymptomatic cows are members of the group known as enterohemorrhagic *Escherichia coli* (EHEC). EHEC are characterized by the production of verotoxins or Shiga toxins and are recognized as the primary cause of hemorrhagic colitis (HC) or bloody diarrhea, which can progress to the potentially fatal hemolytic uremic syndrome (HUS). This group contains the pathogen *E. coli* O157:H7, which was responsible for the waterborne outbreak in Walkerton, Ontario (64). These

organisms are able to colonize the terminal rectum of cattle and can be shed into the feces for up to four weeks (102).

   Escherichia coli O157:H7 can be frequently isolated from cattle feces, and most human cases associated with this organism can be eventually traced back to this source (8, 9). Several studies have been undertaken to identify possible intervention strategies to control the organism on farms. These have included diet, age of cattle, management of manure and fecal slurry, contaminated animal drinking water, and management of pre- and post-weaned calves, all of which have been identified as risk factors for infection and shedding of *E. coli* O157:H7 by cattle (107).

   The studies on the effect of diet on fecal shedding of pathogens are controversial. For example, switching a high-grain diet to a high-quality hay-based diet in cattle has been reported to reduce shedding of acid-resistant *E. coli* (32) and *E. coli* O157:H7 (76) in feces. However, due to the complex nature of the cattle digestive systems, this response has been inconsistent (14, 16, 66). Maintaining cows on pasture does not necessarily guarantee that the milk is free from pathogens. Cows in New Zealand are grazed on pasture year-round and the country is recognized as having one of the most advanced dairy systems in the world. However, their milk is not free from problems. While the sale of raw milk in New Zealand is prohibited, rural populations and farm visitors may frequently consume it. Approximately 10% of notified human cases of Shiga-toxin producing *E. coli* (STEC) infection in New Zealand, mostly due to *E. coli* O157:H7, report consumption of raw milk, although they are also exposed to other risk factors in the farm environment. *Escherichia coli* O157 has been reported, albeit rarely, in fecal samples from dairy and beef cattle, and a single infant case has been associated with contact with raw milk. However, there are insufficient data on the prevalence and numbers of STEC in raw milk to definitively estimate the risk from consumption of raw milk in New Zealand (47).

   In a 14-month study performed on four dairy farms in Wisconsin, *E. coli* O157:H7 could not be isolated from cattle on two farms, but one farm had two separate periods of *E. coli* O157:H7 shedding lasting four months and one month (July to August 1996), while on the remaining farm one animal remained positive for a five-month period (130). Heifers shed O157:H7 strains in feces for one to 16 weeks at levels ranging from 2.0×10^2 to 8.7×10^4 cfu/g. Given that the infectious dose can be as low as 10 cells, contamination of milk with only a small amount of feces may result in potentially dangerous levels of the organism in the milk.

   For example, the dose of *Salmonella* Enteritidis in an outbreak caused by the consumption of ice-cream was determined to be 0.093 cells/g, or six cells in a 65 g serving (61). The samples from this outbreak were found to contain from 0.004 to 0.46 cells/g of *Salmonella* (157). This is within the range of counts of *Salmonella* found in eight raw bulk tank milks in France (3.7–79.2 MPN/ml) using a PCR-based method (39).

   Escherichia coli O157:H7 was also isolated from feed, flies, a pigeon, and water. The authors conclude that *E. coli* O157:H7 is disseminated from a

common source on farms and that strains can persist in a herd for a two-year period. In a similar study carried out in Alberta, Stanford *et al.* (138) monitored shedding of *E. coli* O157:H7 monthly over a one-year period by collecting pooled fecal pats from multiple pens of cattle in five commercial dairies. *Escherichia coli* O157:H7 was isolated from cows on four of the farms and from 13.5% of fecal samples. This indicates that the organism is present on a large percentage of dairy farms. The likelihood of positive isolates was 2.6 times higher in calves and heifers compared with mature cows. However, it is not feasible to rely on monitoring of fecal samples as an indication of *E. coli* O157:H7 carriage, as shedding can be intermittent and is heterogeneous among the population. Also, so-called 'super-shedders' have been identified (19); these are animals that excrete large numbers ($>10^4$ cfu/g) of the bacterium in their feces. The incidence of fecal shedding of *E. coli* O157:H7 on beef and dairy farms is shown in Table 2.1. It is interesting to note that the bacterium could not be detected in fecal samples on any of the farms tested in only one study, which provides further support for the conclusion that the organism is widely disseminated among dairy farms.

Therefore, the potential exists to introduce significant numbers of pathogens into the bulk tank by contamination with a small quantity of feces. However, there is some research that suggests that fecal contamination is not a major source of bacteria in milk (73), but this study is limited in that it looked at only

Table 2.1   Prevalence of *E. coli* O157:H7 on farms

Country	Cattle type	Number of farms	Percentage of farms positive
England	Dairy, suckler and fattener	75	38.7
Wales	Store and finishing cattle	952	21.7
Scotland	Store and finishing cattle	481	18.9
Sweden	Dairy cattle	371	8.9
Denmark	Dairy cattle	60	16.7
Norway	Heifers and milking cows	197	1.0
	Dairy cattle	50	0.0
Spain	Dairy cattle	124	7.0
	Beef cattle	82	1.6
Netherlands	Dairy cattle	678	7.2
	Veal calves	462	9.1
	Dairy cattle	10	70.0
Iran	Dairy cattle	26	3.9
Canada (Saskatchewan)	Feedlot cattle	20	60.0
Canada (Alberta)	Feedlot cattle	84	48.0
US (Ohio)	Dairy cattle	50	8.0
US (Midwest)	Ranch and feedlot cattle	29	72.0
US (Tennessee)	Dairy cattle	30	26.7

Source: from (19).

one farm at one point in time. Other sources of pathogens on the dairy farm include lagoon water, bird droppings and rats (100).

2.1.3   Environmental sources of contamination

Personnel
It is unlikely that personnel contribute significantly as a source of microbial contamination of milk during machine milking, although workers suffering from certain zoonoses, such as Q fever, may pose a potential risk (12).

Aerial contamination
Air is thought to be an insignificant contributor to microbial contamination of raw milk. It has been calculated that airborne bacteria account for <5 cfu/ml of the bacterial load of milk; of these *Bacillus* spores would constitute <1 cfu/ml (7). However, a recent study by Pangloli *et al.* (112) suggests that milking parlor air is a major source of *Salmonella* on the dairy farm.

Water
Water used in the production of milk should be of potable quality. Storage tanks should be protected to prevent access by insects, rodents, birds and other sources of contamination and equipment used to deliver water should be properly cleaned. Problems may arise when untreated water supplies are used to rinse and wash equipment. Such water may contain a diverse array of microorganisms including *Pseudomonas* spp., coliforms, *Bacillus* spp. and numerous other types of bacteria (12). Indeed, Perkins *et al.* (116) have demonstrated the potential for contamination of milk with *E. coli* through wash water. The number of cells contaminating the milk may be small but there is the potential for growth in any residual water remaining on the equipment. Chlorination of the water used in the production of milk is recommended.

Concerns about the use of untreated water, and even of mains supplies, have been heightened in recent years by the increased incidence of *Cryptosporidium parvum*. This is a parasite that causes cryptosporidiosis, a disease of the mammalian intestinal tract, which results in acute, watery and non-bloody diarrhea. Cryptosporidiosis is of particular concern in immunocompromised patients (such as AIDS patients), in whom diarrhea can result in the loss of 10–15 liters of water per day. It is known that oocysts of this parasite can resist chlorination and have been detected in raw milk (65, 79, 135), albeit at low incidence rates (<1%), but their source is undetermined.

The environment is also a major source of *Salmonella* on the dairy farm (112). It was found that milking parlor air (62% positive samples) and bird droppings (63%) were major contamination sources during winter, while feeds (50–58%), water (53–67%), calf bedding (63%), soils (60–63%), milking parlor air (60%) and bird droppings (50%) were the main culprits in the spring. All animal and environmental samples (40–92%) except milking parlor air (25%) and bulk tank milk (29%) were found to contribute significantly to the presence

of *Salmonella* in the summer; whereas the major sources of contamination were feeds (60–71%), cow bedding (59%), cow soils (50%), air (46–71%) and insects (63%) during the fall. Again this illustrates that there are several potential sources of contamination by this pathogen that are difficult to control.

Contamination from milking and storage equipment
Significant contamination of milk can arise from inadequately sanitized surfaces of milking and milk storage equipment. McKinnon *et al.* (94) demonstrated that the total bacterial count of milk may increase by up to 3×10^3 cfu/ml due to milking equipment and by a further 1.5×10^3 cfu/ml from the bulk tank. Organisms can proliferate in milk residues present in crevices, joints, rubber gaskets and dead-ends of badly cleaned milking plant (149). A diversity of bacterial types can be introduced into milk from milk mineral deposits present in milking equipment and arguably the most important of these are the Gram-negative psychrotrophs, which predominate among the microflora that adhere to stainless-steel pipelines used for milk transfer (51). Differences in cleaning regimes and, hence, the level of contamination from farm to farm ensure that considerable variation occurs in the microflora of milking equipment (36). The only real protection against the introduction of bacteria into the milk supply from equipment during milking is adequate sanitation. Variations in temperature and cleaning procedures affect the attachment of bacteria to stainless steel surfaces (17, 141) and the effectiveness of sanitation depends to a large extent on the design of the plant and on other factors such as the hardness of the water supply, which itself can give rise to deposits on milking equipment (111).

Feldmann *et al.* (41) investigated the opportunities for contamination of milking equipment at 31 dairy farms. They found that milk quality was affected by the temperature of the rinsing water, with temperatures of less than 42ºC increasing the likelihood of contamination with *Pseudomonas* spp. and coliforms. In addition, milking clusters kept out of the cluster pick-up between milking had a higher risk of microbial contamination. Contamination of the milking machine and the bulk tank milk with environmental bacterial contaminants was not reduced by various methods of teat cleaning before milking or by post-milking teat disinfection. The type of bedding material influenced bacterial contamination of milking clusters and bulk tank milk. They concluded that microbial contamination of the milking machine was influenced not only by the sanitation procedure but by many other factors, such as milking procedures and the environment of the milking parlor.

It is interesting to note that an outbreak of campylobacteriosis in a farming family, which lasted five months, was associated with the consumption of unpasteurized cows' milk (128). Identical PFGE genotypes of the causative organism, *C. jejuni*, were isolated from human and bovine faeces, and bulk tank milk samples. The source of the pathogen was traced to incompletely sealed rubber liners fitted to a milking machine, as the strains isolated from the rubber liners and the feces of patients had the same PFGE genotype.

Farm bulk tanks do not contribute greatly to the bacterial load of raw milk as they are easy to clean and consequently have much lower bacterial levels than the milk pipeline (36). However, ancillary equipment such as agitators, dipsticks, outlet plugs and cocks can be difficult to clean and these may be a possible source of contamination (12). There is also some speculation that inadequately cleaned bulk tanks can be a source of psychrotrophic sporeformers (97), but by far the most important contribution to microbial load afforded by bulk tanks is potential growth of contaminants during storage (119). The frequency at which milk is collected from the bulk tank can influence bacterial growth. For example, if at collection part of the milk in the bulk tank is 48 hours old or more and the bulk tank milk was not cooled rapidly to 4°C or below, the 'growth potential' of the raw milk microflora is significantly affected (51).

In conclusion, the sources of microbial contamination of milk are many and diverse. This makes it very difficult, if not impossible, to completely eliminate potentially pathogenic bacteria from raw milk.

It has been suggested that the public health problems associated with raw milk are scientifically understood and are controlled on organic dairy farms. However, little has been published on the comparison of the quality and safety of milk produced under organic and conventional farming systems, although organic milk has been implicated in outbreaks of illness related to *E. coli* O157 in California and Denmark (71). It has also been reported that the incidence of subclinical mastitis is greater in herds on organic dairy farms (124).

In many jurisdictions, dairy farms and milk produced on them undergo inspection and testing to ensure that practices such as the addition of water to milk do not occur (54). The milk is also tested for the presence of antibiotics, which discourages the farmers from adulterating their milk.

2.2   Pathogens and milk

Many surveys have detected foodborne pathogens in bulk tank milk and some of these are listed in Table 2.2. Although this table is by no means exhaustive, it illustrates that the prevalence of foodborne pathogens in bulk tank milks varies but there are only a few surveys that have failed to detect the presence of a potential pathogen in any of the milks tested. The position is exacerbated when you consider rates of contamination by all the pathogens. For example, Jayarao *et al*. (69) examined bulk tank milk from 248 dairy herds in Pennsylvania and found that *Campylobacter jejuni* could be isolated from 2% of bulk tanks, Shiga toxin-producing *E. coli* from 2.4%, and *Listeria monocytogenes*, *Salmonella* and *Yersinia enterocolitica* from 2.8%, 6% and 1.2%, respectively. Of the 248 bulk tank milk samples, 32 (13%) contained at least one of the pathogens.

In a similar study, Rohrbach *et al*. (125) reported that the frequency of isolation of foodborne pathogens from 292 bulk tank milks taken from farms in east Tennessee and southwest Virginia was 12.3%, 8.9%, 4.1% and 15.1% for *C.*

Table 2.2 Prevalence of foodborne pathogens in raw milk in North America

Foodborne pathogen	Prevalence (%)	Reference	Foodborne pathogen	Prevalence (%)	Reference
Campylobacter jejuni	0.9	(34)		2.7	(139)
	1.5	(87)		4.6	(70)
	0.4	(95)		12.6	(59)
	1.2	(25)		1.0	(158)
	12.3	(125)		4.9–7.0	(98)
	0.5	(139)		6.5	(153)
	9.2	(70)		2.3	(24)
	2.0	(69)		6.3	(28)
	0.0	(96)		2.8	(69)
Shiga-toxin producing *E. coli*	0.9	(139)	*Salmonella* spp.	4.7	(95)
	3.8	(70)		2.9	(92)
	0.8	(100)		8.9	(125)
	0.0	(24)		0.2	(139)
	0.7	(28)		6.1	(70)
	2.4	(69)		1.5	(59)
	0.2 (by PCR[a])	(75)		2.2	(99)
	12 (milk filters)	(101)		2.6	(153)
				0.0	(24)
Listeria monocytogenes	4.2	(88)		0.0	(28)
	1.3	(38)		6.0	(69)
	5.4	(132)		2.8 (11.8 by PCR[a])	(74)
	4.0	(83)	*Staphylococcus aureus*	34.6	(24)
	1.6	(25)			
	1.9	(40)	*Yersinia enterocolitica*	1.2	(69)
	4.1	(125)		15.1	(125)

[a] Polymerase chain reaction-based assay.

jejuni, *Salmonella* spp., *L. monocytogenes* and *Y. enterocolitica*, respectively. Almost one third (32.5%) of milks sampled contained one pathogen. Neither husbandry, hygiene nor clinical status of the herds, including grade classification of the dairy, milking facilities, barn type, milking hygiene, incidence of clinical mastitis among cows, or the number of cows per farm, were associated with the presence of foodborne pathogens in the milk. Thus, it is impossible to eliminate foodborne pathogens from raw milk merely by following good agricultural practices. Even among the 89 milk producers who used teat disinfection and antibiotic dry cow therapy, and who were classified as having good milking hygiene, 29 (35%) had contaminated bulk tank milks compared to 12 of 39 (31%) farmers with poor milking hygiene.

Thus, the prevalence of foodborne pathogens in milk is influenced by numerous factors such as farm size, number of animals on the farm, hygiene, farm management practices, variation in sampling and types of samples evaluated, differences in detection methodologies used, geographical location and season. However, no husbandry practices have been identified that can guarantee that milk will be free from pathogens.

The isolation of pathogens from milk can also be sporadic. For example, Murinda *et al.* (100) reported the detection of *E. coli* O157:H7 from eight of 30 (26.7%) dairy farms at different sampling times. This makes testing of raw milks for pathogens an unreliable procedure to ensure food safety.

As part of the National Animal Health Monitoring System Dairy 2002 Survey, bulk tank milk samples ($n = 861$) were collected from farms in 21 US states (153). Twenty-two samples (2.6%) were culture-positive for *Salmonella*, and *Listeria monocytogenes* was isolated from 56 (6.5%) of samples, with 52 (6%) of the milks containing serotypes 1/2a, 1/2b and 4b, the most common human clinical isolates. No correlation between somatic cell count (SCC) or total microbial count and the incidence of the pathogens was observed. This highlights the fact that commonly used indicators of milk quality cannot be used to predict the presence of pathogens in milk.

Although dairy cattle are known reservoirs for salmonellae, cattle that are shedding this organism are often asymptomatic and difficult to identify. A dairy herd that was experiencing a sustained, subclinical outbreak of *Salmonella* was monitored for two years (154). The presence of *Salmonella* in feces fluctuated throughout the study and ranged from 8 to 88%. *Salmonella* was detected in 11% of milk samples and in 66% of the milk filters. Weekly bulk milk quality testing results (i.e., bulk tank somatic cell score, standard plate count, preliminary incubation count) were typically well within acceptable ranges, again indicating that milk quality variables had low correlations with the presence of pathogens in the milk. An increase in severe infections caused by *Salmonella* Newport has been reported and this is of concern as many Newport isolates have been shown to be resistant to nine or more antibiotics. Risk factors that may be associated with Newport infection in humans include direct exposure to dairy farms, and ingestion of raw milk and cheese made from unpasteurized raw milk (58). Indeed, *Salmonella* Newport was isolated from about 0.5% of bulk tanks sampled as part of the National Animal Health Monitoring System Dairy 2002 Survey (153).

Campylobacter jejuni is the most frequently identified cause of acute infectious diarrhea in developed countries and can be frequently isolated from bulk tank milk (Table 2.2). One of the main vehicles for infection is the ingestion of contaminated non-pasteurized milk (107), which has resulted in large outbreaks. Dairy cattle get infected through ingestion of water and feeds contaminated with manure, and using manure as a fertilizer is considered a risk factor for the occurrence of campylobacteriosis. *Campylobacter jejuni* is also an infrequent cause of mastitis in dairy cows and can be shed from the udder into milk of asymptomatic cows (57). Direct excretion of *C. jejuni* into milk by clinically healthy cows has also been described and implicated in human enteritis following consumption of the contaminated milk (109).

The prevalence of *Listeria monocytogenes* in bulk tank milk has been reported to range from about 1% to 12% (Table 2.2). This organism is widely found in the environment, especially on plants and in soils. It has also been isolated from mammals, birds, fish, crustaceans and insects. In cattle, *L.*

monocytogenes can cause neurological disease, abortion or asymptomatic infections. Healthy but infected animals shed *Listeria* in feces, and fecal contamination of pastures or vegetables has been implicated as a source of contamination for humans and ruminants. *Listeria monocytogenes* can grow in a wide range of temperature and pH conditions, including in refrigerated raw milk and in low-quality silage with a pH of 4.5; it can cause mastitis in cows, and it can be shed in milk of asymptomatic cows, potentially resulting in high levels of the organism in raw milk (6).

The microbiological quality of 1097 samples of unpasteurized milk at point of sale from 242 retail outlets in England and Wales showed that potentially pathogenic bacteria could be isolated from 41 milks sold at 28 retail outlets (27). Salmonellae were isolated from 0.5% of milks, whereas *E. coli* O157 and *Campylobacter* spp. were present in 0.3% and 1.7% of samples, respectively. *Staphylococcus aureus* was present at a count above the legal limit in England and Wales (500 cfu/ml) in 1% of milks. Lancefield group C or G streptococci were present at counts exceeding 100 cfu/ml in 0.2% of samples. Although group C and G streptococci are not considered harmful to humans, two other groups, A and D, can be transmitted to humans via food, with infectious doses of <1000 and 10 million cells, respectively. The milks containing either salmonellae or *E. coli* O157 all came from different retail outlets. Twenty-nine of the 41 milks found to contain potential pathogens had satisfactory bacterial counts, indicating once more that good overall hygiene did not guarantee the absence of pathogens. The study concluded that the continuing availability of unpasteurized milk on retail sale constitutes an unacceptable risk to public health.

Several other human pathogens have been isolated from raw milk, including *Staphylococcus aureus*, *Yersinia enterocolitica*, *Coxiella burnettii*, *Bacillus cereus*, etc. (1, 51, 54, 107, 126). Of concern is the recent finding that human norovirus strains can be present in cattle and are present in feces (91). Norovirus causes approximately 90% of epidemic, non-bacterial outbreaks of gastro-enteritis worldwide and is responsible for 50% of all foodborne outbreaks of gastroenteritis in the US. Norovirus affects people of all ages and is transmitted by fecally contaminated food or water and by person-to-person contact.

2.3   Limitations of raw milk testing as an indicator of safety

Conventional measures of raw milk quality such as total bacterial count and somatic cell count are not indicative of the presence of pathogens. Whereas total bacterial count gave the highest correlation with on-farm hygiene practices (68), there is no correlation between this indicator and the presence of pathogens in raw milk (31, 39). Thus, pathogens can be isolated from milk with a low bacterial count produced under hygienic conditions.

The use of testing for particular pathogens to ensure safety relies on the premise that if pathogens are not detectable in raw milk or the animals from which it is derived, then the milk should be safe for human consumption.

However, several issues confound the use of product testing. These include the following:

- Milk contamination occurs sporadically so it is difficult to develop reliable sampling strategies.
- Contamination may not be evenly distributed in the milk; for example, it is known that a large proportion of organisms present in milk are associated with the fat.
- The numbers of organisms present in the milk may be below the numbers that can be detected by the method used but still be large enough to produce illness due to their low infectious dose.
- Extremely low numbers of organisms that are below the limit of detection may be present in the product but can then grow to levels that are unacceptable after testing.
- It is impossible to test for all the pathogens that may be present in the milk.

2.4   Outbreaks of illness associated with the consumption of raw milk

Because pathogens cannot be entirely eliminated from milk, outbreaks due to its consumption continue to occur. In developed countries, the incidence of outbreaks where milk has been identified as the vehicle of infection is between 1.5% and 6.5% (26, 48, 60, 80).

De Buyser *et al*. (26) analyzed the proportion of milk-borne diseases due to *Salmonella* spp., *Staph. aureus*, *L. monocytogenes* and pathogenic *E. coli* reported in seven countries between 1980 and 1997. Particular attention was given to whether the milk involved was heat-treated or not. Milk and milk products were implicated in 1–5% of the total bacterial outbreaks; however, details about the type of product and milk involved were not always provided. Of the 60 outbreaks and four single cases where milk and milk products were implicated, milk was the vehicle in 39.1% of cases. Overall, 32.8% of the products were made from pasteurized milk, 58.4% from raw or unpasteurized milk, and 18.8% from milk where the heat treatment it received was not specified. *Salmonella* spp. were responsible for 29 outbreaks, *L. monocytogenes* for 10 outbreaks and four well-documented single cases, pathogenic *E. coli* for 11 outbreaks, and *Staph. aureus* for 10 outbreaks. Data obtained from the French surveillance system between 1992 and 1997 revealed 69 documented outbreaks for which milk and milk products were confirmed as the vehicle by the isolation of the etiologic agent. In this case, raw milk and raw milk products accounted for 48% of outbreaks. *Staphylococcus aureus* was by far the most frequent pathogen associated with these outbreaks (85.5%), followed by *Salmonella* (10.1%).

Interestingly, de Buyser *et al*. (26) pointed out that pasteurized milk was associated with more cases of illness (Table 2.3), but this can be explained by the fact that more people consume this product and that, in all cases, the

Table 2.3   Distribution of cases according to etiological agent and milk type

Etiological agent (no. of outbreaks)	Heat treatment of milk			Total no. of cases (deaths)
	Raw	Pasteurized	Not specified	
S. aureus (10)	207	878	69	1154 (0)
Salmonella (29)	4116 (10)	16809 (10)	100	21025 (20)
L. monocytogenes (14)	173 (37)	265 (36)	42 (6)	480 (79)
E. coli (11)	90 (1)	439	15	544 (1)
Total (deaths)	4586 (48)	18451 (46)	226 (6)	23263 (100)

Source: from (26).

outbreak was due to deficiencies in the pasteurization process which meant that raw milk was actually being consumed or the organism was introduced post-pasteurization. Table 2.4 shows a breakdown of the outbreaks linked to pasteurized milk in the US between 1960 and 2000 and the cause of the contamination. There have been several well-documented cases indicating that pasteurizer faults or post-pasteurization contamination is responsible for outbreaks associated with pasteurized milk (22, 49, 151).

Between 1880 and 1907, an average of 29 outbreaks of milk-borne diseases were reported each year in the United States (20). The adoption of pasteurization in the US in 1938 had a dramatic effect, with Headrick *et al*. (60) reporting 46 outbreaks of milk-borne diseases in the 19-year period from 1973 to 1992, an average of 2.4 per year. Before 1938, an estimated 25% of all foodborne and

Table 2.4   Causes of pasteurized milk outbreaks in the US (1960–2000)

Year	Pathogen	Total no. ill (confirmed)	Mechanism of contamination
1966	*Shigella flexneri*	97 (97)	Post pasteurization
1975	*Salmonella* Newport	49 (49)	Unknown
1976	*Yersinia enterocolitica*	38 (38)	Post pasteurization
1978	*S.* Typhimurium	23 (23)	Post pasteurization
1982	*Y. enterocolitica*	172 (172)	Unknown
1983	*Listeria monocytogenes*	49 (40)	Unknown
1984	*S.* Typhimurium	16 (16)	Inadequate pasteurization
1985	*S.* Typhimurium	>150,000 (>16,000)	Post pasteurization
1986	*Campylobacter jejuni*	33 (8)	Inadequate pasteurization
1994	*L. monocytogenes*	45 (11)	Post pasteurization
1995	*Y. enterocolitica*	10 (10)	Post pasteurization
2000	*S.* Typhimurium	93 (38)	Post pasteurization

Source: from (108).

Fig. 2.1   Reported outbreaks of disease suspected or confirmed to be associated with unpasteurized milk in the United States, 1993–2006. From (81).

waterborne outbreaks of disease were associated with milk, but by 2001 the percentage of such outbreaks associated with milk was estimated at <1%. A review of foodborne diseases reported to the CDC that were suspected or confirmed to be associated with unpasteurized milk or milk products between 1993 and 2006 identified 68 outbreaks, an average of 5.2 per year (81) (Fig. 2.1). Although some of this increase may be due to improvements in detection and surveillance, the data clearly show that disease associated with the consumption of raw milk is still an important public health issue.

There is no food that is absolutely free of risk but there are very few foods where there is a simple and practical way to significantly decrease risk without compromising quality. Pasteurization of milk is one such process, which, in its modern form, was introduced to control the pathogen *Coxiella burnetii*. Indeed, there is a vast literature demonstrating the efficacy of pasteurization in reducing milk-borne illness and an equally weighty volume of literature documenting illness produced through the consumption of raw milk (107, 126, 128). In England and Wales from 1985 to 1989, the great majority of outbreaks of milk-borne illness were associated with the consumption of raw milk, although it accounted for less than 1% of sales. In Scotland a similar situation existed until the sale of unpasteurized milk was prohibited in 1983. After this time the incidence of disease associated with liquid milk dropped significantly and was further reduced when legislation was introduced in 1986 to prohibit farm workers from receiving untreated milk as part of their wages (15, 51). The higher number of cases observed in England and Wales may simply reflect differences in population levels or may be due to the fact that the sale of unpasteurized milk was still allowed. The decrease in cases observed in England and Wales in 1988 may have been due to an increase in vigilance within the industry following an outbreak of salmonellosis linked to infant formula in 1985, which involved 62 cases and one death.

Further evidence to indicate the hazard associated with consumption of raw milk was produced during a study of foodborne infections conducted in Ontario between 1979 and 1985 (144). Cases of salmonellosis, campylobacteriosis and yersiniosis were more prevalent in rural areas and it was concluded that raw milk played an important role in these infections. A further study of foodborne illness in Ontario between 1997 and 2001 indicated that, in patients where the food associated with illness could be determined, 6.9% of *Campylobacter* infections, 1.6% of *Salmonella* cases, 3.5% of verotoxigenic *E. coli* illness, and 4.5% of cases of yersiniosis were attributed to raw milk (80). Of all the cases where the vehicle for infection was identified, 4.3% were due to consumption of contaminated raw milk, even though the sale of raw milk in the province is illegal. From 2005 to 2007, 92 cases of illness caused by raw milk or cheese made from unpasteurized milk were reported in Ontario. Campylobacteriosis (61 cases) was the most common illness reported, and six cases of *E. coli* O157:H7 occurred in 2005. Three cases of *E. coli* O157:H7 in Simcoe County were directly linked to the consumption of raw milk. The Simcoe-Muskoka District Health Unit was informed that the families received the contaminated milk from an individual who routinely distributed milk from a parked vehicle (2). It is not hard to imagine how this figure would be impacted if sales of raw milk were allowed.

Another index of the dynamic nature of milk-borne illness is the change observed in the etiological agents with time. In the mid-twentieth century the main illnesses associated with the consumption of milk were brucellosis and tuberculosis. These have been eradicated as milk-borne illnesses in developed countries, mainly through herd certification programs, the installation of refrigerated bulk tanks for collection of milk on farms and the introduction of pasteurization. The majority of present-day milk-borne illnesses are attributable to *Salmonella* spp., *Campylobacter* spp., *E. coli* and *L. monocytogenes* among others and are associated with the consumption of raw milk or pasteurized milk that has either received an inadequate heat-treatment or has been contaminated after heating. These epidemiological changes have been brought about by the adoption of new milk production, processing and distribution practices. Other factors, for example the changing characteristics of microorganisms and demographic changes such as the ageing population and increase in numbers of immunocompromised individuals, will ensure that this dynamism will continue (37).

Since 2005, several outbreaks of disease, including salmonellosis, *E. coli* O157:H7 infections and campylobacteriosis, related to consumption of unpasteurized milk have been reported. As a result of one notable outbreak, 18 cases of infection with *E. coli* O157:H7 occurred in Oregon and Washington states in 2005. The people affected were mostly children aged below 14 years. Five patients, aged 1–13 years, were hospitalized, and four contracted hemolytic uremic syndrome, which results in severe renal failure and can be fatal. The cases were linked to raw milk from a dairy participating in a cow-share program in Washington (30), though it should be noted that this farm was not licensed.

In 2007, 29 cases of *Salmonella* Typhimurium infection were associated with consumption of raw milk sold at a farm in Pennsylvania. Sixteen of the 29 patients were aged below 7 years (85). Farms in Pennsylvania that hold raw-milk permits undergo twice-monthly milk testing for coliforms and standard plate counts and monthly testing for growth inhibitors and somatic cell counts. There is also an annual inspection and culture of raw milk for *Salmonella*, *Campylobacter*, *E. coli* O157 and *L. monocytogenes*, and annual herd skin testing for *Mycobacterium bovis* and *Brucella*. Despite these measures, it is apparent that consumers cannot be assured that certified raw milk is free of pathogens.

As of 2004, at least 27 US states permitted some form of raw-milk sales to the public, including sales at dairies, farmers' markets, or through purchase of 'cow shares'. Certain states also allow public sales of raw milk but for pet food only. In Pennsylvania, the number of dairies with raw-milk permits increased from 42 in 2005 to 75 in 2007. During 2006–2007, three clusters of illness from *Campylobacter* were associated with consumption of raw milk from three different Pennsylvania dairies. During the same period, the Pennsylvania Department of Agriculture announced raw-milk recalls from three other dairies after finding *L. monocytogenes* in milk samples. However, no human illness was associated with this milk.

At least 87 people became ill in Kansas in two separate outbreaks of campylobacteriosis towards the end of 2007. In both outbreaks, illness was associated with consumption of raw milk or raw-milk products (81). In 2008, an outbreak of campylobacteriosis in California was associated with consumption of unpasteurized milk supplied from a farm operating a cow-share program. One of the patients consequently developed Guillain–Barré syndrome (81). This is an autoimmune disease affecting the peripheral nervous system, usually triggered by an acute infection. It is frequently severe and usually exhibits as an ascending paralysis noted by weakness in the legs that spreads to the upper limbs and the face along with complete loss of deep tendon reflexes. With prompt treatment, the majority of patients will recover. However, death may occur if severe pulmonary complications arise. This outbreak has been, in part, responsible for the introduction of Senate Bill 201, which gives raw-milk dairies the alternative of producing a Hazard Analysis Critical Control Point (HACCP) plan for each critical process in the production of raw milk on the dairy farm. The plan would have to be approved by the California Department of Food and Agriculture (CDFA) and the State Department of Public Health (DPH), California. A dairy producing raw milk under such an alternative HACCP plan would have to have its raw milk tested twice per week by a state-accredited laboratory for certain bacteria, with results reported to the CDFA, and to the DPH upon request. The raw milk must also be tested monthly for pathogens. It is interesting to note that intrastate sale of raw milk is legal in Washington, Pennsylvania, Kansas, and California.

In December 2007 a listeriosis outbreak, which resulted in the deaths of three elderly men and an unborn baby, was linked to the consumption of pasteurized milk produced by Whittier Farms. This outbreak was unusual in that it involved

milk that was bottled in glass bottles for home delivery to customers. The outbreak strain was isolated from coffee-flavored milk produced at the dairy as well as in samples taken from the floor of the plant and in equipment used after pasteurization, indicating that the contaminant was introduced after pasteurization. The same strain was also found in seven unopened bottles of milk that were on shelves at the retail store next to the plant (121). Bottling machines are notoriously difficult to clean and the level of bacterial contamination found in bottled milk is inevitably higher than found in milks packed in cartons (56).

What is of particular concern about these outbreaks is the age of many of the patients. For example, in the Pennsylvania outbreak of *Salmonella* Typhimurium, 16 of the 29 patients were aged under 7 years. Caregivers should carefully consider unsubstantiated claims concerning the health benefits of raw milk for infants and children, because infants and children are dependent on these people to make safe dietary decisions for them. Clearly, this has had devastating consequences for many children. These individuals do not buy milk 'directly from a farm' but are exposed to its risks and deserve to be protected.

It should also be noted that dairy farmers are not immune to infection through the consumption of raw milk. While it appears that regular consumers of raw milk may develop partial immunity to enteric pathogens over time, they remain susceptible to large doses, where numbers of the pathogen in the milk are high, or to new strains to which they have not been previously exposed. For example, when *Salmonella* Muenster first affected Ontario dairy herds, many dairy farm families developed illness (144). Also, Schildt *et al.* (128) described an outbreak of campylobacteriosis of five months' duration in a farming family. The outbreak was traced to the consumption of unpasteurized milk contaminated with *C. jejuni*. Six members of the family acquired the illness, and two had several episodes of diarrhea within the five-month period. Identical genotypes of *C. jejuni* were isolated from human and bovine feces, and bulk tank milk samples. The contamination was probably due to incompletely sealed rubber liners fitted to a milking machine shortly before the outbreak started, allowing fecal material to contaminate the milk.

A list of known outbreaks associated with milk in North America between 2000 and 2007 is shown in Table 2.5. These outbreaks indicate that raw milk is not inherently safe.

2.5   Routes of transmission of foodborne pathogens

According to studies on the extent of secondary transmission for *E. coli* O157 and other pathogens, the initially reported foodborne illnesses in the outbreak may represent only a small fraction of the eventual number of cases that include asymptomatic infections and secondary infections spread by person-to-person contact among household members of infected persons and other close contacts.

Table 2.5 Outbreaks of illness in North America between 2001 and 2009 associated with consumption of milk

Year	Organism	Source	State or province	No. of cases
2009	*C. jejuni*	Raw milk (or exposed to someone who became ill after consuming raw milk)	WI	13
2009	*Campylobacter*	Raw milk (cow share)	CO	11
2009	*Campylobacter*	Raw milk	PA	6
2008	*Campylobacter*	Raw milk	PA	7
2008	*E. coli*	Raw milk	CA	15 (1 in intensive care)
2008	*E. coli*	Raw milk	CT	5
2007	*Listeria monocytogenes*	Pasteurized milk	MA	4 (3 deaths)
2007/08	*Campylobacter jejuni*	Raw milk	WA	5
2007	*C. jejuni*	Raw milk	KS	19
2007	*C. jejuni*	Raw milk	GA	3 families; 1 child hospitalized
2007	*Salmonella* Typhimurium	Raw milk	PA	8
2007	*Campylobacter*	Raw milk	NC	1
2007	*C. jejuni*	Raw milk	UT	>15 (1 hospitalized)
2007	*Yersinia/Listeria*	Raw milk	ON	2 children (1 hospitalized)
2007	*Salmonella*	Raw milk	PA	2
2006	*Campylobacter*	Raw milk	ON	6
2006	*Escherichia coli* O157:H7	Raw milk	WA	2 children
2006	*E. coli* O157:H7	Raw milk/colostrum	CA	4 children (aged 7–10)
2006	*E. coli* O157:H7/ *Campylobacter*	Raw milk	ON	2 (15 year old hospitalized)
2006	Unidentified	Raw milk	ON	2 (farm family)
2006	*C. jejuni*	Raw milk	OH	3 (2 children)
2005	*E. coli* O157:H7	Raw milk	WA	6 (aged 5–14)
2005	*E. coli* O157:H7	Raw milk sold from truck	ON	6
2003	*C. jejuni*	Raw milk	UT	13 (aged 11–50)
2002–03	*S.* Typhimurium	Raw milk	IL, IN, OH, TN	62 (2 children hospitalized)
2001	*C. jejuni*	Raw milk from organic farm involved in cow leasing program	WI	75 (aged 2–63)

Source: http://www.milkfacts.info/Milk%20Microbiology/Disease%20Outbreaks.htm; www.foodsafety.ksu.edu/articles/1138/Raw_Milk_Outbreak_Table.pdf

For *E. coli* O157 a large proportion (72%) of infections are asymptomatic, exposure to low doses can result in infection, and reported secondary transmission rates are of the order of 4–16% (113, 129). Other highly infectious enteric pathogens, including *Shigella*, *Salmonella* and *Campylobacter*, can spread from person to person after being introduced into a community through water, food or other sources with secondary attack rates of 4–20% (117). Children have a two- to eight-fold greater chance of acquiring secondary infections than adults (117). Lee and Middleton (80) reviewed enteric illness in Ontario between 1997 and 2001 and reported that 6% of infections were acquired through person-to-person contact. Thus, people who contract gastroenteritis from the consumption of raw milk can spread the infection to people who did not drink the milk.

Dairy farmers may also act as asymptomatic carriers of disease and act as a reservoir of infection. Wilson *et al*. (161) took fecal samples from 335 dairy farm residents and 1458 cattle on 80 farms and tested them for Verocytotoxin (VT)-producing *Escherichia coli* (VTEC). Residents were also tested for antibodies to the verotoxin produced by the pathogen and antibodies to the organism itself. Residents and cattle on farms with VTEC-positive persons or *E. coli* O157:H7-positive cattle were retested. Twenty-one persons (6.3%) on 16 farms (20.8%) and 46% of cattle on 100% of the farms had VTEC in fecal samples. Human VTEC isolates included *E. coli* O157:H7 and eight other serotypes, four of which were present in cattle on the same farms. More persons had antibodies to VT1 (41%) than to O157 LPS (12.5%). The presence of the antibody against the organism in the blood of the people tested was associated with isolation of *E. coli* O157:H7 on the farm. However, people who shed the pathogen in their feces did not show symptoms of the disease. Similarly, Silvestro *et al*. isolated VTEC O157 from stool samples of four (1.1%) farm workers in Italy (131) who also did not show signs of infection.

The people most at risk of acquiring gastroenteric infections by drinking raw milk are the very young, the elderly, pregnant women, those already suffering from an illness, or immuno-compromised persons. However, anyone can be affected, including healthy young adults. For example, Blaser *et al.* (10) documented an outbreak of campylobacteriosis among 19 of 31 college students who consumed unpasteurized milk during a farm visit.

Furthermore, the consequences of acquiring a milk-borne infection may not be limited to the usual symptoms of diarrhea, vomiting, nausea, fever, abdominal cramps, etc. A significant number of people who suffer gastroenteritis can go on to develop more serious symptoms. Infections due to *Salmonella* (in particular *S.* Enteritidis and *S.* Typhimurium), *Campylobacter jejuni*, *Shigella* spp. and *Yersinia enterocolitica* appear to give rise to a number of chronic joint diseases, which include reactive arthritis, Reiter's syndrome and ankylosing spondylitis (137). Symptoms commonly begin approximately 7–30 days after an intestinal illness. The knee is often infected along with other peripheral joints. The duration of symptoms varies considerably, but in most individuals symptoms subside in less than six months. However, some individuals may take in excess

of one year to recover fully, and a significant proportion of affected persons suffer persistent or relapsing illnesses.

In addition to environmental factors, genetic factors play a significant role in the development of these joint diseases following exposure to a triggering organism. Approximately 6–10% of white Americans, 2–3% of African Americans and 1% of Japanese people carry the gene for susceptibility. Approximately 2–3% of all exposed individuals develop one of the reactive arthritides (18). However, approximately 20% of exposed susceptible individuals will develop one of the reactive arthritides, with Reiter's syndrome occurring 10 times less frequently than reactive arthritis (3). Detailed epidemiological investigations of several foodborne disease outbreaks suggest that these rates may underestimate the true number of cases and the associated economic impact of reactive arthritides due to foodbome disease. Following an outbreak of *Salmonella* Enteritidis, 15.7% of those affected subsequently developed one of the reactive arthritides (86). In another outbreak due to *Salmonella* Typhimurium, 16.4% of those ill developed reactive arthritis and 9.4% developed conjunctivitis (134). Nearly 40% of those with reactive arthritis had symptoms that persisted for over one year. The attack rate for reactive arthritis among exposed persons in these outbreaks is seven to eight times higher than the generally reported rate (2–3%).

Campylobacter jejuni infections can lead to Guillain–Barré syndrome (GBS), which is an acute, progressive neuropathy characterized by paralysis, pain, muscular weakness and distal sensory loss (136). The disease progresses rapidly, sometimes within the course of a single day, although symptoms may take several weeks to develop. Case-fatality rates reported in the literature range from 2% to 8% (43). Although the syndrome can probably arise from a number of viral and bacterial infections, *C. jejuni* is recognized as the most common preceding infection. Epidemiological studies have highlighted the relationship between GBS and prior *C. jejuni* infection. A case-control study in England and Wales between 1992 and 1994 showed that 26% of patients with GBS or Miller–Fisher syndrome (a variant of GBS) had suffered from campylobacteriosis (122). Another study of patients with GBS showed that one year after the onset of disease, 8% of the patients had died, 4% were still bed-ridden and 9% were still unable to walk without assistance. The estimated annual cost of GBS in the US is $1.7 billion, including $0.2 billion (14%) in direct medical costs and $1.5 billion (86%) in indirect costs. The mean cost per patient with GBS was $318,966 (43).

Several complications may arise in susceptible individuals following infection with *Listeria monocytogenes*. These include septicemia, meningitis, encephalitis, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion (second or third trimester) or stillbirth (35, 143). Approximately 5% of people who contract a *L. monocytogenes* infection develop endocarditis. The infective dose of *L. monocytogenes* is unknown but is believed to vary with the strain and susceptibility of the victim. From cases contracted through raw or supposedly pasteurized milk, it is safe to assume that

in susceptible persons, fewer than 1000 cells may cause disease. The overall mortality rate is 20–30%; of all pregnancy-related cases, 22% resulted in fetal loss or neonatal death, but mothers usually survive. However, among patients who have acquired listerial meningitis, the mortality rate may be as high as 70%; the corresponding rate for patients with septicemia is 50%, and for perinatal/ neonatal infections the rate is greater than 80%. It is interesting to note that approximately 5–15% of adults shed this bacterium in their stool without showing signs of infection.

In about 2–7% of cases, particularly among children under five years of age, an enterohemorrhagic *E. coli* infection can give rise to hemolytic uremic syndrome (HUS), which can result in severe kidney disease (145). In the United States, HUS is the principal cause of acute kidney failure in children, and most cases of hemolytic uremic syndrome are caused by *E. coli* O157:H7. With aggressive treatment more than 90% survive the acute phase. About 9% may develop end-stage renal disease, approximately one-third of persons with hemolytic uremic syndrome have abnormal kidney function many years later, and a few require long-term dialysis. Another 8% of persons with hemolytic uremic syndrome have other lifelong complications, such as high blood pressure, seizures, blindness, paralysis, and the effects of having part of their colon removed. The overall mortality rate from HUS is 5–15%, with older children and adults having a worse prognosis.

Thus, what, in the first instance, appears to be a bout of sickness and diarrhea can evolve into illnesses with chronic and possibly fatal consequences. As well as the social impact, these sequelae can impose a severe financial burden on the healthcare system.

2.6   Antimicrobial properties of milk

It has been suggested that raw milk contains several antimicrobial systems that inhibit the growth of pathogens. These are outlined in Table 2.6, which indicates that they can retain activity in pasteurized milk.

Pitt *et al.* (120) investigated the antimicrobial activity of raw milk against three pathogens: *Staph. aureus*, *Salmonella* Enteritidis and *L. monocytogenes*. They found that populations of *Staph. aureus* and *Salmonella* Enteritidis increased in both raw and pasteurized milk at 37ºC but levels declined after 32 hours in raw milk. The decline in counts was less in pasteurized milk, and the authors concluded that this was due to the antimicrobial activity present in raw milk being inactivated by the heat treatment. Final counts of *Staph. aureus* and *Salmonella* Enteritidis after 72 hours were approximately 100- and 1000-fold higher, respectively, in pasteurized milk than in raw milk. *Listeria mono-cytogenes* inoculated into raw milk at 37ºC to give an initial bacterial concentration of approximately 10^4 cfu/ml multiplied at a reduced rate for approximately 12 hours and then rapidly lost viability. Fifty-six hours after the inoculation of raw milk, no viable cells of *L. monocytogenes* were detected,

Table 2.6   Antimicrobial systems in raw milk

Milk component	Role in milk	Effect of pasteurization	Reference
Lactoferrin	An iron-binding protein; scavenger of iron, thereby providing antibacterial effects by limiting the availability of free iron required for bacterial proliferation	Unheated and pasteurized bovine lactoferrin have similar antibacterial properties	(114)
Lacto-peroxidase	A milk enzyme which, in conjunction with other enzymes, contributes to the bacteriostatic properties of milk. To be effective, both hydrogen peroxide and thiocyanate ions must be present; both of these chemicals are not endogenous to milk but are byproducts of other bacterial metabolic activity	Retains 70% of activity when heated to 72ºC for 15 s, the minimum HTST pasteurization process. Other studies have shown that it retains almost all its activity at HTST temperature, but loses 90% of activity after 38 min at 71ºC or 4 min at 75ºC	(21, 55, 90)
Lysozyme	Active primarily against Gram-positive bacteria. In conjunction with lactoferrin has bactericidal effects	Greater than 75% of activity retained after heating at 80ºC for 15 s	(42)
Bovine immuno-globulin	Transfers immunity against bovine pathogens to calves; may provide some lactogenic immunity in the gut. Most immunoglobulins are carried in the colostrum, which is generally not consumed	No loss in activity during batch pasteurization for 30 min at 62.7ºC; retains 59–76% of activity after HTST pasteurization	(82)
Bacteriocins	Antimicrobial peptides that are produced by bacteria that may be present in milk. They have a narrow spectrum of antimicrobial activity affecting mainly Gram-positive bacteria	Heat stable and retain activity after pasteurization	(89, 155)
Oligo-saccharides	Competitively bind to pathogens to prevent adherence of pathogens to target mucosal receptors	Heat stable	(103)
Xanthine oxidase	An enzyme linked with flavor changes in milk during storage. Claimed to have antimicrobial properties	Retains enzymatic activity after heating at 73ºC for 7 min or at 80ºC for about 50 s	(29, 140)

Source: from (81).

which indicated that the raw milk had 'killed' the organism. However, this study has little relevance as the incubation temperature was well above that used to store raw milk and only *L. monocytogenes* would be able to grow at the low temperatures encountered in bulk tank milk. The study also does not indicate that raw milk would have an advantage to combat infection if consumed. The methodology is also flawed as only a single strain of each of the three pathogens was investigated.

There may be differences in the way strains respond and this is demonstrated by the study of Doyle and Roman (34). They monitored the survival of eight *Campylobacter* strains in unpasteurized milk at 4ºC. The ability of the organisms to survive varied greatly, with the most tolerant strain showing a <2-log cycle decrease in viable cells after 14 days, while the most sensitive strain showed a >6-log cycle decrease after 7 days. One strain was still recoverable 21 days after the inoculation of the milk. They concluded that, because of the possible persistence of *C. jejuni* in raw grade A milk, pasteurization of milk was necessary.

In another study, the effect of raw milk on the growth of *L. monocytogenes* at 15ºC was investigated (45). Six strains of *L. monocytogenes* isolated from raw milk were used to evaluate the inhibitory effect of the lactoperoxidase system. After 65 hours in static conditions, populations of *L. monocytogenes* in pasteurized milk increased by 2 to 3.8 log cycles, depending on the strain. In raw milk, under the same conditions, populations increased by 0.8 to 2.3 log cycles. Addition of thiocyanate and hydrogen peroxide to raw milk to activate the lactoperoxidase system enhanced its inhibitory effect, indicated by the fact that three strains were unable to grow and the populations of the other strains increased by 0.7 to 1.3 log cycles. However, again this study has limited relevance to raw milk under the storage conditions used in practice, i.e. approximately 4ºC. The growth of the organism was inhibited slightly after 65 hours (2.7 days), a long storage time for raw milk, but was not completely prevented. It is also unlikely that thiocyanate and hydrogen peroxide would normally be added to the bulk tank to stimulate the lactoperoxidase system, although activation of the enzyme system can be achieved in the presence of streptococci.

One study has tested the efficacy of lactoperoxidase in milk at 4ºC and 8ºC (4). Following three days of storage at 4ºC, there was little change in counts of *E. coli* O157:H7 or *Y. enterocolitica*, there was a reduction in counts of both *Salmonella* Enterica and *Aeromonas hydrophila* by about 1 and 2 log cycles (or 10- and 100-fold), respectively; whereas *C. jejuni* counts decreased by about 4 log cycles. Again, there were drawbacks to this experiment in that raw milk was not the medium used and single strains of the pathogens were tested. The study involved UHT-treated skim milk to which pure lactoperoxidase was added along with thiocyanate and hydrogen peroxide.

2.6.1   Lactoferrin

Lactoferrin, a protein found in milk and many mucosal secretions such as tears and saliva, is known to exert a broad-spectrum activity against bacteria, fungi,

protozoa and viruses by binding with iron, which is essential for the growth of microorganisms. This results in a bacteriostatic effect (110). Many foodborne pathogens are also inhibited by lactoferricin B, a bactericidal peptide produced by gastric pepsin digestion of lactoferrin (5). Lactoferrin is not greatly affected by pasteurization (Table 2.6) and so raw milk does not have an advantage over the heat-treated product. In addition the activity is bacteriostatic, so the microorganisms are not killed, but rather their growth is inhibited. As many of the pathogens encountered in milk will not grow at the refrigeration temperatures encountered in the bulk tank, this agent has little relevance for promoting milk safety.

2.6.2   Lactoperoxidase

Lactoperoxidase is an enzyme found in milk that has antimicrobial and antioxidant properties. None of the research conducted to date has demonstrated that this system works effectively to control pathogens in raw milk at refrigeration temperatures. As an enzyme, its activity is influenced by temperature, so it would be less active at the refrigeration temperatures used to store raw milk. It is also known that lactoperoxidase is very heat stable and would be present in significant quantities in pasteurized milk (Table 2.6). It requires the addition of two chemicals, thiocyanate and hydrogen peroxide, to activate the antimicrobial activity, so its use in milk to control pathogens is limited.

2.6.3   Lysozyme

Lysozyme is an enzyme that degrades bacterial cell walls and mainly affects Gram-positive bacteria. It has little effect on enteric pathogens such as *E. coli* O157:H7 and *Salmonella* (115). Being an enzyme, its activity is affected by temperature, so again it would have limited activity at refrigeration temperatures. The enzyme can survive pasteurization and would be present in heat-treated milk (Table 2.6).

2.6.4   Bovine immunoglobulins

The importance of milk immunoglobulins or antibodies to the newborn has been reviewed by Wheeler *et al.* (159), who state that levels of these proteins are much higher in colostrum than in milk. However, the activity of immunoglobulins is not completely destroyed by pasteurization (Table 2.6). The scientific literature provides limited evidence to support the claim that components of raw milk provide immunity to humans. However, most of the work in this area involves the use of antibodies and other components that are purified from milk (23, 78). There is also no scientific evidence to suggest that bovine somatic cells (white blood cells) found in milk influence the immunity of humans to disease.

2.6.5   Bacteriocins and probiotics

Bacteriocins are small peptides produced by bacteria that inactivate other closely related bacteria by damaging their cell membranes. Many lactic acid bacteria isolated from milk are capable of producing bacteriocins (123) but it is unlikely that they would reach levels necessary for the producton of these molecules in refrigerated milk as they would not grow. In a survey of raw milk in France, counts of the lactic acid bacteria associated with bacteriocin production were low. *Lactococcus* counts varied between 690 and 3600 cfu/ml, *Lactobacillus* counts ranged from 180 to 3000 cfu/ml and *Enterococcus* counts ranged from 74 to 400 cfu/ml.

It has also been claimed that raw milk contains probiotic bacteria, again mainly lactic acid bacteria. Probiotic microorganisms are those that confer a health benefit to their target host. They need to be digested in high numbers to survive transit through the intestinal tract, so again it is unlikely that the 'probiotic' bacteria in raw milk would be present at high enough numbers to become an established part of the microflora of the human intestine. To produce a beneficial effect, probiotics must be consumed at levels approximately 1000- to 10,000-fold higher than those found in raw milk.

2.6.6   Xanthine oxidase

Xanthine oxidase is an enzyme. It is present in milk and can contribute to activation of the lactoperoxidase system by supplying it with hydrogen peroxide. It may also kill bacteria through production of a range of products that cause oxidative stress of the bacterial cells (159). Again, the activity of the enzyme would be limited at low temperatures and the enzyme can survive pasteurization (Table 2.6).

2.6.7   Other potential antimicrobial agents

Other molecules are present in milk that have antimicrobial activity. These include the proteins β-defensins, cathelicidins and angiogenin, which are found in human milk and some at elevated levels in colostrum. The roles that these proteins play in bovine milk are still to be verified (159).

2.7   References

1. ANON. 1994. Monograph on the significance of pathogenic microorganisms in raw milk. *International Dairy Federation*: 1–215.
2. ANON. November 28, 2006, Avoid unpasteurized, raw milk and milk products. Media release, York Region Health Services, Ontario. [Online.] http://www.york.ca/ Publications/News/2006/November+28,+2006++Avoid+unpasteurized,+raw+milk+ and+milk+products.htm
3. ARCHER, D. L., and F. E. YOUNG. 1988. Contemporary issues: diseases with a food vector. *Clinical Microbiology Reviews* **1**: 377–398.

4. ARQUES, J. L., E. RODRIGUEZ, M. NUNEZ, and M. MEDINA. 2008. Inactivation of Gram-negative pathogens in refrigerated milk by reuterin in combination with nisin or the lactoperoxidase system. *European Food Research and Technology* **227**: 77–82.

5. BELLAMY, W., M. TAKASE, H. WAKABAYASHI, K. KAWASE, and M. TOMITA. 1992. Antibacterial spectrum of lactoferricin B, a potent bactericidal peptide derived from the N-terminal region of bovine lactoferrin. *Journal of Applied Microbiology* **73**: 472–479.

6. BEMRAH, N., M. SANAA, M. H. CASSIN, M. W. GRIFFITHS, and O. CERF. 1998. Quantitative risk assessment of *Listeria monocytogenes* in soft cheeses made from raw milk. *Preventive Veterinary Medicine* **37**: 129–145.

7. BENHAM, C. L., and J. W. EGDELL. 1970. Levels of airborne bacteria in milking premises. *Journal of the Society of Dairy Technology* **23**: 91–94.

8. BESSER, T. E., D. D. HANCOCK, L. C. PRITCHETT, E. M. MCRAE, D. H. RICE, and P. I. TARR. 1997. Duration of detection of *Escherichia coli* O157:H7 in cattle. *Journal of Infectious Diseases* **175**: 726–729.

9. BESSER, T. E., B. L. RICHARDS, D. H. RICE, and D. D. HANCOCK. 2001. *Escherichia coli* O157:H7 infection of calves: infectious dose and direct contact transmission. *Epidemiology and Infection* **127**: 555–560.

10. BLASER, M., E. SAZIE, and L. P. J. WILLIAMS. 1987. The influence of immunity on raw milk-associated *Campylobacter* infection. *Journal of the American Medical Association* **257**: 43–46.

11. BOOR, K. J. 1997. Pathogenic microorganisms of concern to the dairy industry. *Dairy, Food and Environmental Sanitation* **17**: 714–717.

12. BRAMLEY, A. J., and C. H. MCKINNON. 1990. The microbiology of raw milk, in R. K. Robinson (ed.), *Dairy Microbiology*, 2nd edn, vol. 1, pp. 163–208. Elsevier Applied Science, London.

13. BRAMLEY, A. J., C. H. MCKINNON, R. T. STAKER, and D. C. SIMPKIN. 1984. The effect of udder infection on the bacterial flora of the bulk milk of ten dairy herds. *Journal of Applied Bacteriology* **57**: 317–323.

14. BUCHKO, S. J., R. A. HOLLEY, W. O. OLSON, V. P. GANNON, and D. M. VEIRA. 2000. The effect of different grain diets on fecal shedding of *Escherichia coli* O157:H7 by steers. *Journal of Food Protection* **63**: 1467–1474.

15. BURT, R., and S. WELLSTEED. 1991. Food safety and legislation in the dairy industry. *Journal of the Society of Dairy Technology* **44**: 80–86.

16. CALLAWAY, T. R., R. C. ANDERSON, T. S. EDRINGTON, K. J. GENOVESE, K. M. BISCHOFF, T. L. POOLE, Y. S. JUNG, R. B. HARVEY, and D. J. NISBET. 2004. What are we doing about *Escherichia coli* O157:H7 populations in cattle? *Journal of Animal Science* **82**: E93–E99.

17. CARPENTIER, B., and O. CERF. 1993. Biofilms and their consequences, with particular reference to hygiene in the food industry. *Journal of Applied Bacteriology* **75**: 499–511.

18. CAST. 1994. *Foodborne Pathogens: Risks and Consequences*. Council for Agricultural Science and Technology, Ames, IA.

19. CHASE-TOPPING, M., D. GALLY, C. LOW, L. MATTHEWS, and M. WOOLHOUSE. 2008. Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. *Nature Reviews Microbiology* **6**: 904–912.

20. CHIN, J. 1982. Raw milk: a continuing vehicle for the transmission of infectious disease agents in the United States. *Journal of Infectious Diseases* **146**: 440–441.

21. CLAEYS, W. L., L. R. LUDIKHUYZE, A. M. VAN LOEY, and M. E. HENDRICKX. 2001. Inactivation kinetics of alkaline phosphatase and lactoperoxidase, and denaturation

kinetics of β-lactoglobulin in raw milk under isothermal and dynamic temperature conditions. *Journal of Dairy Research* **68**: 95–107.

22. CLARK, A., S. MORTON, P. WRIGHT, J. CORKISH, F. J. BOLTON, and J. RUSSELL. 1997. A community outbreak of Vero cytotoxin producing *Escherichia coli* O157 infection linked to a small farm dairy. CDR Review. *Communicable Disease Report* **7**: R206–R211.

23. CROSS, M. L., and H. S. GILL. 2000. Immunomodulatory properties of milk. *British Journal of Nutrition* **84**: 81–89.

24. D'AMICO, D. J., E. GROVES, and C. W. DONNELLY. 2008. Low incidence of foodborne pathogens of concern in raw milk utilized for farmstead cheese production. *Journal of Food Protection* **71**: 1580–1589.

25. DAVIDSON, R. J., D. W. SPRUNG, C. E. PARK, and M. K. RAYMAN. 1989. Occurrence of *Listeria monocytogenes*, *Campylobacter* spp. and *Yersinia enterocolitica* in Manitoba raw milk. *Canadian Institute of Food Science and Technology Journal* **22**: 70–74.

26. DE BUYSER, M.-L., B. DUFOUR, M. MAIRE, and V. LAFARGE. 2001. Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. *International Journal of Food Microbiology* **67**: 1–17.

27. DE LOUVOIS, J., and A. RAMPLING. 1998. One fifth of samples of unpasteurized milk are contaminated with bacteria. *British Medical Journal* **316**: 7131.

28. DE RUE, K., K. GRIJSPEERDT, and L. HERMAN. 2004. A Belgian survey of hygiene indicator bacteria and pathogenic bacteria in raw milk and direct marketing of raw milk farm products. *Journal of Food Safety* **24**: 17–36.

29. DEMOTT, B. J., and O. A. PRAEPANITCHAI. 1978. Influence of storage, heat, and homogenization upon xanthine oxidase activity of milk. *Journal of Dairy Science* **61**: 164–167.

30. DENNY, J., M. BHAT, and K. ECKMANN. 2008. Outbreak of *Escherichia coli* O157:H7 associated with raw milk consumption in the Pacific Northwest. *Foodborne Pathogens and Disease* **5**: 321–328.

31. DESMASURES, N., F. BAZIN, and M. GUEGUEN. 1997. Microbiological composition of raw milk from selected farms in the Camembert region of Normandy. *Journal of Applied Microbiology* **83**: 53–58.

32. DIEZ-GONZALES, F., T. R. CALLAWAY, M. G. KIZOULIS, and J. B. RUSSELL. 1998. Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. *Science* **281**: 1666–1668.

33. DONNELLY, L. S., and F. F. BUSTA. 1981. Anaerobic sporeforming microorganisms in dairy products. *Journal of Dairy Science* **64**: 161–166.

34. DOYLE, M. P., and D. J. ROMAN. 1982. Prevalence and survival of *Campylobacter jejuni* in unpasteurized milk. *Applied and Environmental Microbiology* **44**: 1154–1158.

35. DREVETS, D. A., and M. S. BRONZE. 2008. *Listeria monocytogenes*: epidemiology, human disease, and mechanisms of brain invasion. *FEMS Immunology and Medical Microbiology* **53**: 151–165.

36. DRUCE, R. G., and S. B. THOMAS. 1972. Bacteriological studies on bulk milk collection. Pipeline milking plants and bulk tanks as sources of bacterial contamination of milk. *Journal of Applied Bacteriology* **35**: 253–270.

37. EYLES, M. J. 1995. Trends in foodborne disease and implications for the dairy industry. *Australian Journal of Dairy Technology* **50**: 10–14.

38. FARBER, J. M., G. W. SANDER, and S. A. MALCOLM. 1988. The prevalence of *Listeria* spp. in raw milk in Ontario. *Canadian Journal of Microbiology* **34**: 95–100.

39. FARES, A. 2007. Quantitative risk assessment model of human salmonellosis linked to the consumption of Camembert cheese made from raw milk. AgroParisTech, Paris.

40. FEDIO, W. M., and H. JACKSON. 1990. Incidence of *Listeria monocytogenes* in raw milk in Alberta. *Canadian Institute of Food Science and Technology Journal* **23**: 236–238.

41. FELDMANN, M., A. ZIMMERMANN, and M. HOEDEMAKER. 2006. Influence of milking technique, milking hygiene and environment on microbial contamination of milking machine. *Deutsche Tierarztliche Wochenschrift* **113**: 274–281.

42. FOX, P. F., and A. L. KELLY. 2006. Indigenous enzymes in milk: overview and historical aspects. *International Dairy Journal* **16**: 500–516.

43. FRENZEN, P. D. 2008. Economic cost of Guillain–Barré syndrome in the United States. *Neurology* **71**: 21–27.

44. GALTON, D. M., L. G. PETERSSON, and W. G. MERRILL. 1986. Effects of premilking udder preparation practices on bacterial counts in milk and on teats. *Journal of Dairy Science* **69**: 260–266.

45. GAY, M., and A. AMGAR. 2005. Factors moderating *Listeria monocytogenes* growth in raw milk and in soft cheese made from raw milk. *Lait* **85**: 153–170.

46. GIBSON, H., L. A. SINCLAIR, C. M. BRIZUELA, H. L. WORTON, and R. G. PROTHEROE. 2008. Effectiveness of selected premilking teat-cleaning regimes in reducing teat microbial load on commercial dairy farms. *Letters in Applied Microbiology* **46**: 295–300.

47. GILBERT, S., R. LAKE, A. HUDSON, and P. CRESSEY. 2007. Risk profile: Shiga-toxin producing *Escherichia coli* in raw milk. Institute of Environmental Science and Research Limited, Christchurch Science Centre, Christchurch, New Zealand.

48. GILLESPIE, I. A., G. K. ADAK, S. J. O'BRIEN, and F. J. BOLTON. 2003. Milkborne general outbreaks of infectious intestinal disease, England and Wales, 1992–2000. *Epidemiology and Infection* **130**: 461–468.

49. GOH, S., C. NEWMAN, M. KNOWLES, F. J. BOLTON, V. HOLLYOAK, S. RICHARDS, P. DALEY, D. COUNTER, H. R. SMITH, and N. KEPPIE. 2002. *E. coli* O157 phage type 21/28 outbreak in North Cumbria associated with pasteurized milk. *Epidemiology and Infection* **129**: 451–457.

50. GRANT, M. A., C. A. EKLUND, and S. C. SHIELDS. 1995. Monitoring dairy silage for five bacterial groups with potential for human pathogenesis. *Journal of Food Protection* **58**: 879–883.

51. GRIFFITHS, M. W. 2004. Milk and unfermented milk products, in B. M. Lund, A. C. Baird-Parker, and G. W. Gould (eds), *The Microbiological Safety and Quality of Food*, vol. 1, pp. 507–534, Aspen Publishers, Gaithersburg, MD.

52. GRIFFITHS, M. W. 2006. *Mycobacterium paratuberculosis*, in Y. Motarjemi and M. Adams (eds), *Emerging Foodborne Pathogens*, pp. 522–556, Woodhead Publishing, Cambridge.

53. GRIFFITHS, M. W. 2009. *Mycobacterium paratuberculosis*, in C. D. W. Blackburn and P. J. McClure (eds), *Foodborne Pathogens: Hazards, Risk Analysis and Control*, 2nd edn, pp. 1060–1118, Woodhead Publishing, Cambridge.

54. GRIFFITHS, M. W. 2007. The safety of milk and dairy foods: Is there anything new we should be aware of? *Australian Journal of Dairy Technology* **62**: 67–75.

55. GRIFFITHS, M. W. 1986. Use of milk enzymes as indices of heat treatment. *Journal of Food Protection* **49**: 696–705.

56. GRIFFITHS, M. W., and J. D. PHILLIPS. 1986. The application of the preincubation test in

commercial dairies. *Australian Journal of Dairy Technology* **41**: 71–79.

57. GUDMUNDSON, J., and J. M. CHIRINO-TREJO. 1992. A case of bovine mastitis caused by *Campylobacter jejuni*. *Zentralblatt für Veterinärmedizin. Reihe B* **40**: 326–328.

58. GUPTA, A., J. FONTANA, C. CROWE, B. BOLSTORFF, A. STOUT, S. VAN DUYNE, M. P. HOESKSTRA, J. M. WHICHARD, T. J. BARRETT, and F. J. ANGULO. 2003. Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. *Journal of Infectious Diseases* **188**: 1707–1716.

59. HASSAN, L., H. O. MOHAMMED, P. L. MCDONOUGH, and R. N. GONZALEZ. 2000. A cross-sectional study on the prevalence of *Listeria monocytogenes* and *Salmonella* in New York dairy herds. *Journal of Dairy Science* **83**: 2441–2447.

60. HEADRICK, M. L., S. KORANGY, N. H. BEAN, F. J. ANGULO, S. F. ALTEKRUSE, M. E. POTTER, and K. C. KLONTZ. 1998. The epidemiology of raw milk-associated foodborne disease outbreaks reported in the United States, 1973 through 1992. *American Journal of Public Health* **88**: 1219–1221.

61. HENNESSY, T. W., C. W. HEDBERG, L. SLUTSKER, K. E. WHITE, J. M. BESSER-WIEK, M. E. MOEN, J. FELDMAN, W. W. COLEMAN, L. M. EDMONSON, K. L. MACDONALD, and M. T. OSTERHOLM. 1996. A national outbreak of *Salmonella enteritidis* infections from ice-cream. *New England Journal of Medicine* **334**: 1281–1286.

62. HERLIN, A. H., and A. CHRISTIANSSON. 1993. Cheese-blowing anaerobic spores in bulk milk from loose-housed and tied dairy cows. *Milchwissenschaft* **48**: 686–690.

63. HILLERTON, J. E., A. J. BRAMLEY, R. T. STAKER, and C. H. MCKINNON. 1995. Patterns of intramammary infection and clinical mastitis over a five year period in a closely monitored herd applying mastitis control measures. *Journal of Dairy Research* **62**: 39–50.

64. HOLME, R. 2003. Drinking water contamination in Walkerton, Ontario: positive resolutions from a tragic event. *Water Science and Technology* **47**: 1–6.

65. HOSKIN, J. C., and R. E. WRIGHT. 1991. *Cryptosporidium*: an emerging concern for the food industry. *Journal of Food Protection* **54**: 53–57.

66. HOVDE, C. J., P. R. AUSTIN, K. A. CLOUD, C. J. WILLIAMS, and C. W. HUNT. 1999. Effect of cattle diet on *Escherichia coli* O157:H7 acid resistance. *Applied and Environmental Microbiology* **65**: 3233–3235.

67. HUSU, J. R., J. T. SEPPANEN, S. K. SIVELA, and A. L. RAURAMAA. 1990. Contamination of raw milk by *Listeria monocytogenes* on dairy farms. *Journal of Veterinary Medicine Series B* **37**: 268–275.

68. HUTCHISON, M. L., D. J. I. THOMAS, A. MOORE, D. R. JACKSON, and I. OHNSTAD. 2005. An evaluation of raw milk microorganisms as markers of on-farm hygiene practices related to milking. *Journal of Food Protection* **68**: 764–772.

69. JAYARAO, B. M., S. C. DONALDSON, B. A. STRALEY, A. A. SAWANT, N. V. HEGDE, and J. L. BROWN. 2006. A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. *Journal of Dairy Science* **89**: 2451–2458.

70. JAYARAO, B. M., and D. R. HENNING. 2001. Prevalence of foodborne pathogens in bulk tank milk. *Journal of Dairy Science* **84**: 2157–2162.

71. JENSEN, C., S. ETHELBERG, A. GERVELMEYER, E. M. NIELSEN, K. E. P. OLSEN, and K. MOLBAK. 2006. First general outbreak of verotoxin-producing *Escherichia coli* O157 in Denmark. *European Surveillance* **11**: 55–58.

72. JOHNS, C. K. 1962. The coliform count of milk as an index of udder cleanliness. Presented at the 16th International Dairy Congress, Copenhagen.

73. KAGKLI, D. M., M. VANCANNEYT, P. VANDAMME, C. HILL, and T. M. COGAN. 2007. Contamination of milk by enterococci and coliforms from bovine faeces. *Journal of Applied Microbiology* **103**: 1393–1405.

74. KARNS, J. S., J. S. VAN KESSEL, B. J. MCCLUSKEY, and M. L. PERDUE. 2005. Prevalence of *Salmonella enterica* in bulk tank milk from US dairies as determined by Polymerase Chain Reaction. *Journal of Dairy Science* **88**: 3475–3479.

75. KARNS, J. S., J. S. VAN KESSEL, B. J. MCCLUSKY, and M. L. PERDUE. 2007. Incidence of *Escherichia coli* O157:H7 and *E. coli* virulence factors in US bulk tank milk as determined by polymerase chain reaction. *Journal of Dairy Science* **90**: 3212–3219.

76. KEEN, J. E., G. A. UHLICH, and R. O. ELDER. 1999. Effects of hay and grain-based diets on fecal shedding in naturally-acquired enterohemorrhagic *E. coli* (EHEC) O157:H7 shedding in beef feedlot cattle. 80th Conference Research Workers in Animal Diseases, Chicago.

77. KIM, S. G., E. H. KIM, C. J. LAFFERTY, and E. DUBOVI. 2005. *Coxiella burnettii* in bulk tank milk samples, United States. *Emerging Infectious Diseases* **11**: 619–621.

78. KORHONEN, H., P. MARNILA, and H. S. GILL. 2000. Milk immunoglobulins and complement factors. *British Journal of Nutrition* **84**: 75–80.

79. LABERGE, I., and M. W. GRIFFITHS. 1996. Prevalence, detection and control of *Cryptosporidium parvum* in food. *International Journal of Food Microbiology* **32**: 1–26.

80. LEE, M. B., and D. MIDDLETON. 2003. Enteric illness in Ontario, Canada, from 1997 to 2001. *Journal of Food Protection* **66**: 953–961.

81. LEJEUNE, J. T., and P. J. RAJALA-SCHULTZ. 2009. Unpasteurized milk: A continued public health threat. *Clinical Infectious Diseases* **48**: 93–100.

82. LI-CHAN, E., A. KUMMER, J. N. LOSSO, D. D. KITTS, and S. NAKAI. 1995. Stability of bovine immunoglobulins to thermal treatment and processing. *Food Research International* **28**: 9–16.

83. LIEWEN, M. B., and M. W. PLAUTZ. 1988. Occurrence of *Listeria monocytogenes* in raw milk in Nebraska. *Journal of Food Protection* **51**: 840–841.

84. LIMOND, A., and M. W. GRIFFITHS. 1991. Use of the Bactofoss to assess quality of raw and pasteurized milk. *International Dairy Journal* **1**: 167–182.

85. LIND, L., J. REESER, K. STAYMAN, M. DEASY, M. MOLL, A. WELTMAN, V. URDANETA, and S. OSTROFF. 2008. *Salmonella* Typhimurium infection associated with raw milk and cheese consumption – Pennsylvania 2007. *Journal of the American Medical Association* **299**: 402–404.

86. LOCHT, H. E., E. KIHLSTROM, and F. D. LINDSTROM. 1993. Reactive arthritis after *Salmonella* among medical doctors – study of an outbreak. *Journal of Rheumatology* **20**: 845–848.

87. LOVETT, J., D. W. FRANCIS, and J. M. HUNT. 1983. Isolation of *Campylobacter jejuni* from raw milk. *Applied and Environmental Microbiology* **46**: 459–462.

88. LOVETT, J., D. W. FRANCIS, and J. M. HUNT. 1987. *Listeria monocytogenes* in raw milk: detection, incidence, and pathogenicity. *Journal of Food Protection* **50**: 188–192.

89. MARINEZ, B., D. BRAVO, and A. RODRIGUEZ. 2005. Consequences of the development of nisin-resistant *Listeria monocytogenes* in fermented dairy products. *Journal of Food Protection* **68**: 2383–2388.

90. MARKS, N. E., A. S. GRANDISON, and M. J. LEWIS. 2001. Challenge testing of the lactoperoxidase system in pasteurized milk. *Journal of Applied Microbiology* **91**: 735–741.

91. MATTISON, K., A. SHUKLA, A. COOK, F. POLLARI, R. FRIENDSHIP, D. KELTON, S. BIDAWID,

and J. M. FARBER. 2007. Human noroviruses in swine and cattle. *Emerging Infectious Diseases* **13**: 1184–1188.

92. MCEWEN, S. A., S. W. MARTIN, R. C. CLARKE, S. E. TAMBLYN, and J. J. MCDERMOTT. 1988. The prevalence, incidence, geographical distribution, antimicrobial sensitivity patterns and plasmid profiles of milk filter *Salmonella* isolates from Ontario dairy farms. *Canadian Journal of Veterinary Research* **52**: 18–22.

93. MCKINNON, C. H., and G. L. PETTIPHER. 1983. A survey of sources of heat-resistant bacteria in milk with particular reference to psychrotrophic spore-forming bacteria. *Journal of Dairy Research* **50**: 163–170.

94. MCKINNON, C. H., G. J. ROWLANDS, and A. J. BRAMLEY. 1990. The effect of udder preparation before milking and contamination from the milking plant on bacterial numbers in bulk milk of eight dairy herds. *Journal of Dairy Research* **57**: 307–318.

95. MCMANUS, C., and J. M. LANIER. 1987. *Salmonella*, *Campylobacter jejuni*, and *Yersinia enterocolitica* in raw milk. *Journal of Food Protection* **50**: 51–55.

96. MEDEIROS, D. T., S. A. SATTAR, J. M. FARBER, and C. D. CARRILLO. 2008. Occurrence of *Campylobacter* spp. in raw and ready-to-eat foods and in a Canadian food service operation. *Journal of Food Protection* **71**: 2087–2093.

97. MEER, R. R., J. M. BAKER, F. W. BODYFELT, and M. W. GRIFFITHS. 1991. Psychrotrophic *Bacillus* spp. in fluid milk products. A review. *Journal of Food Protection* **54**: 969–979.

98. MURAOKA, W., C. GAY, D. KNOWLES, and M. BORUCKI. 2003. Prevalence of *Listeria monocytogenes* subtypes in bulk milk of the Pacific Northwest. *Journal of Food Protection* **66**: 1413–1419.

99. MURINDA, S. E., L. T. NGUYEN, S. J. IVEY, B. E. GILLESPIE, R. A. ALMEIDA, F. A. DRAUGHON, and S. P. OLIVER. 2002. Molecular characterization of *Salmonella* spp. isolated from bulk tank milk and cull dairy cow fecal samples. *Journal of Food Protection* **65**: 1100–1105.

100. MURINDA, S. E., L. T. NGUYEN, S. J. IVEY, B. E. GILLESPIE, R. A. ALMEIDA, F. A. DRAUGHON, and S. P. OLIVER. 2002. Prevalence and molecular characterization of *Escherichia coli* O157:H7 in bulk tank milk and fecal samples from cull cows: A 12-month survey of dairy farms in East Tennessee. *Journal of Food Protection* **65**: 752–759.

101. MURPHY, B. P., M. MURPHY, J. F. BUCKLEY, D. GILROY, M. T. ROWE, D. MCCLEERY, and S. FANNING. 2005. In-line milk filter analysis: *Escherichia coli* O157 surveillance of milk production holdings. *International Journal of Hygiene and Environmental Health* **208**: 407–413.

102. NAYLOR, S. W., J. C. LOW, T. E. BESSER, A. MAHAJAN, G. J. GUNN, M. C. PEARCE, I. J. MCKENDRICK, D. G. E. SMITH, and D. L. GALLY. 2003. Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infection and Immunity* **71**: 1505–1512.

103. NEWBURG, D. S., G. M. RUIZ-PALACIOS, and A. L. MORROW. 2005. Human milk glycans protect infants against enteric pathogens. *Annual Reviews of Nutrition* **25**: 37–58.

104. NIGHTINGALE, K. K., Y. H. SCHUKKEN, C. R. NIGHTINGALE, E. D. FORTES, A. J. HO, Z. HER, Y. T. GROHN, P. L. MCDONOUGH, and M. WIEDMANN. 2004. Ecology and transmission of *Listeria monocytogenes* infecting ruminants and the farm environment. *Applied and Environmental Microbiology* **70**: 4458–4467.

105. NISKANEN, A., L. KOIRANEN, and K. ROINE. 1978. Staphylococcal enterotoxin and thermonuclease production during induced bovine mastitis and the clinical reaction of enterotoxin in udders. *Infection and Immunity* **19**: 493–498.

106. OLDE RIEKERINK, R. G. M., H. W. BARKEMA, D. F. KELTON, and D. T. SCHOLL. 2008. Incidence rate of clinical mastitis on Canadian dairy farms. *Journal of Dairy Science* **91**: 1366–1377.

107. OLIVER, S. P., B. M. JAYARAO, and R. A. ALMEIDA. 2005. Foodborne pathogens in milk and the dairy farm environment: Food safety and public health implications. *Foodborne Pathogens and Disease* **2**: 115–129.

108. OLSEN, S. J., M. YING, M. F. DAVIS, M. DEASY, B. HOLLAND, L. IAMPIETRO, C. M. BAYSINGER, F. SASSANO, L. D. POLK, B. GORMLEY, M. J. HUNG, K. PILOT, M. ORSINI, S. VAN DUYNE, S. RANKIN, C. GENESE, E. A. BRESNITZ, J. SMUCKER, M. MOLL, and J. SOBEL. 2004. Multidrug-resistant *Salmonella* Typhimurium infection from milk contaminated after pasteurization. *Emerging Infectious Diseases* **10**: 932–935.

109. ORR, K. E., N. F. LIGHTFOOT, P. R. SISSON, B. A. HARKIS, J. L. TWEDDLE, P. BOYD, A. CARROLL, C. J. JACKSON, D. R. A. WAREING, and R. FREEMAN. 1995. Direct milk excretion of *Campylobacter jejuni* in a dairy cow causing cases of human enteritis. *Epidemiology and Infection* **114**: 15–24.

110. ORSI, N. 2004. The antimicrobial activity of lactoferrin: current status and perspectives. *Biometals* **17**: 189–196.

111. PALMER, J. 1980. Contamination of milk from the milking environment. *IDF Bulletin*: 16–21.

112. PANGLOLI, P., Y. DJE, O. AHMED, C. A. DOANE, S. P. OLIVER, and F. A. DRAUGHON. 2008. Seasonal incidence and molecular characterization of *Salmonella* from dairy cows, calves, and farm environment. *Foodborne Pathogens and Disease* **5**: 87–96.

113. PARRY, S. M., and R. L. SALMON. 1998. Sporadic STEC O157 infection: secondary household transmission in Wales. *Emerging Infectious Diseases* **4**: 657–661.

114. PAULSSON, M. A., U. SVENSSON, A. R. KISHORE, and A. S. NAIDU. 1993. Thermal behavior of bovine lactoferrin in water and its relation to bacterial interaction and antibacterial activity. *Journal of Dairy Science* **76**: 3711–3720.

115. PAYNE, K. D., S. P. OLIVER, and P. M. DAVIDSON. 1994. Comparison of EDTA and apo-lactoferrin with lysozyme on the growth of foodborne pathogenic and spoilage bacteria. *Journal of Food Protection* **57**: 62–65.

116. PERKINS, N. R., D. F. KELTON, K. E. LESLIE, K. J. HAND, G. MACNAUGHTON, and O. BERKE. 2007. An analysis of the relationship between wash water quality and bulk tank milk quality on Ontario dairy farms. *Journal of Dairy Science* **90**: 330.

117. PERRY, S., M. DE LA LUZ SANCHEZ, and J. PARSONNET. 2005. Household transmission of gastroenteritis. *Emerging Infectious Diseases* **11**: 1093–1096.

118. PHILLIPS, J. D., and M. W. GRIFFITHS. 1986. Factors contributing to the seasonal variation of *Bacillus* spp. in pasteurised dairy products. *Journal of Applied Bacteriology* **61**: 275–285.

119. PHILLIPS, J. D., and M. W. GRIFFITHS. 1990. Pasteurized dairy products: The constraints imposed by environmental contamination, in J. O. Nriagu and M. S. Simmons (eds), *Food Contamination from Environmental Sources*, pp. 387–456, John Wiley & Sons, New York.

120. PITT, W. M., T. J. HARDEN, and R. R. HULL. 2000. Investigation of the antimicrobial activity of raw milk against several foodborne pathogens. *Milchwissenschaft* **55**: 249–252.

121. PRITZKER, F., and R. RUOHONEN. 2008. Food poisoning law blog. [Online.] http://foodpoisoning.pritzkerlaw.com/archives/cat_whittier_farms_lawsuit.html

122. REES, J. H., S. E. SOUDAIN, N. A. GREGSON, and R. A. C. HUGHES. 1995. *Campylobacter jejuni* infection and Guillain–Barré syndrome. *New England Journal of Medicine*

333: 1374–1379.

123. RODRIGUEZ, A., B. GONZALEZ, P. GAYA, M. NUNEZ, and M. MEDINA. 2000. Diversity of bacteriocins produced by lactic acid bacteria isolated from raw milk. *International Dairy Journal* **10**: 7–15.

124. ROESCH, M., M. G. DOHERR, W. SCHAREN, M. SCHALLIBAUM, and J. W. BLUM. 2007. Subclinical mastitis in dairy cows in Swiss organic and conventional production systems. *Journal of Dairy Research* **74**: 86–92.

125. ROHRBACH, B. W., F. A. DRAUGHON, P. M. DAVIDSON, and S. P. OLIVER. 1992. Prevalence of *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Salmonella* in bulk tank milk: risk factors and risk of human exposure. *Journal of Food Protection* **55**: 93–97.

126. RYSER, E. T. 2004. Public health concerns, in E. H. Marth, and J. L. Steele (eds), *Applied Dairy Microbiology*, pp. 397–545, Marcel Dekker, New York.

127. SANAA, M., B. POUTREL, J. L. MENARD, and F. SERIEYS. 1993. Risk factors associated with contamination of raw milk by *Listeria monocytogenes* in dairy farms. *Journal of Dairy Science* **76**: 2891–2898.

128. SCHILDT, M., S. SAVOLAINEN, and M. L. HANNINEN. 2006. Long-lasting *Campylobacter jejuni* contamination of milk associated with gastrointestinal illness in a farming family. *Epidemiology and Infection* **134**: 401–405.

129. SETO, E. Y. W., J. A. SOLLER, and J. M. J. COLFORD. 2007. Strategies to reduce person-to-person transmission during widespread *Escherichia coli* O157:H7 outbreak. *Emerging Infectious Diseases* **13**: 860–866.

130. SHERE, J. A., K. J. BARTLETT, and C. W. KASPAR. 1998. Longitudinal study of *Escherichia coli* O157:H7 dissemination on four dairy farms in Wisconsin. *Applied and Environmental Microbiology* **64**: 1390–1399.

131. SILVESTRO, L., M. CAPUTO, S. BLANCATO, L. DECASTELLI, A. FIORAVANTI, R. TOZZOLI, S. MORABITO, and A. CAPRIOLI. 2004. Asymptomatic carriage of verocytotoxin-producing *Escherichia coli* O157 in farm workers in Northern Italy. *Epidemiology and Infection* **132**: 915–919.

132. SLADE, P. J., D. L. COLLINS-THOMPSON, and F. FLETCHER. 1988. Incidence of *Listeria* species in Ontario raw milk. *Canadian Institute of Food Science and Technology Journal* **21**: 425–429.

133. SLAGHUIS, B. A., T. DE VRIES, and J. G. P. VERHEIJ. 1991. Bacterial load of different materials which can contaminate milk during production. *Milchwissenschaft* **46**: 574–578.

134. SMITH, J. L. 1994. Arthritis and foodborne bacteria. *Journal of Food Protection* **57**: 935–941.

135. SMITH, J. L. 1993. *Cryptosporidium* and *Giardia* as agents of foodborne disease. *Journal of Food Protection* **56**: 451–461.

136. SMITH, J. L. 1996. Determinant that may be involved in virulence and disease in *Campylobacter jejuni*. *Journal of Food Safety* **16**: 105–139.

137. SMITH, J. L., S. A. PALUMBO, and I. WALLS. 1993. Relationship between foodborne bacterial pathogens and the reactive arthritides. *Journal of Food Safety* **13**: 209–236.

138. STANFORD, K., D. CROY, S. J. BACH, G. L. WALLINS, H. ZAHIRODDINI, and T. A. MCALLISTER. 2005. Ecology of *Escherichia coli* O157:H7 in commercial dairies in southern Alberta. *Journal of Dairy Science* **88**: 4441–4451.

139. STEELE, M. L., W. B. MCNAB, C. POPPE, M. W. GRIFFITHS, C. SHU, S. A. DEGRANDIS, L. C. FRUHNER, C. A. LARKIN, J. A. LYNCH, and J. A. ODUMERU. 1997. Survey of Ontario bulk

tank raw milk for food-borne pathogens. *Journal of Food Protection* **60(11)**: 1341–1346.

140. STEVENS, C. R., T. M. MILLAR, J. G. CLINCH, J. M. KANCZLER, T. BODAMYALI, and D. R. BLAKE. 2000. Antibacterial properties of xanthine oxidase in human milk. *Lancet* **356**: 829–830.

141. STONE, L. S., and E. A. ZOTTOLA. 1985. Effect of cleaning and sanitizing on the attachment of *Pseudomonas fragi* to stainless steel. *Journal of Food Science* **50**: 951–956.

142. SUTHERLAND, A. D., and R. MURDOCH. 1994. Seasonal occurrence of psychrotrophic *Bacillus* species in raw milk, and studies on the interactions with mesophilic *Bacillus* sp. *International Journal of Food Microbiology* **21**: 279–292.

143. SWAMINATHAN, B., and P. GERNER-SMIDT. 2007. The epidemiology of human listeriosis. *Microbes and Infection* **9**: 1236–1243.

144. TAMBLYN, S. E. 1987. Zoonoses on the family farm. Canadian Veterinary Journal 28: 260.

145. TAYLOR, C. M. 2008. Enterohaemorrhagic *Escherichia coli* and *Shigella dysenteriae* type 1-induced haemolytic uraemic syndrome. *Pediatric Nephrology* **23**: 1425–1431.

146. TE GIFFEL, M. C., R. R. BEUMER, B. A. SLAGHUIS, and F. M. ROMBOUTS. 1995. Occurrence and characterization of (psychrotrophic) *Bacillus cereus* on farms in the Netherlands. *Netherlands Milk and Dairy Journal* **49**: 125–138.

147. THOMAS, S. B., and R. G. DRUCE. 1971. Bacteriological quality of alternate day collected farm bulk tanks milk. *Dairy Science Abstracts* **33**: 339–342.

148. THOMAS, S. B., R. G. DRUCE, and M. JONES. 1971. Influence of production conditions on the bacteriological quality of refrigerated farm bulk tank milk – a review. *Journal of Applied Bacteriology* **34**: 659–677.

149. THOMAS, S. B., and B. F. THOMAS. 1977. The bacterial content of milking machines and pipeline milking plants. Part II of a review. *Dairy Industries International* **42**: 19–25.

150. TOLLE, A. 1980. The microflora of the udder. *IDF Bulletin*: 4–10.

151. UPTON, P., and J. E. COIA. 1994. Outbreak of *Escherichia coli* O157 infection associated with pasteurised milk supply. *Lancet* **344**: 1015.

152. VALLE, J., E. GOMEZ-LUCIA, S. PIRIZ, and S. VADILLO. 1991. Staphylococcal enterotoxins and toxic shock syndrome toxin-1 production during induced goat mastitis. *Journal of Food Protection* **54**: 267–271.

153. VAN KESSEL, J. S., J. S. KARNS, L. GORSKI, B. J. MCCLUSKEY, and M. L. PERDUE. 2004. Prevalence of salmonellae, *Listeria monocytogenes*, and fecal coliforms in bulk tank milk on US dairies. *Journal of Dairy Science* **87**: 2822–2830.

154. VAN KESSEL, J. S., J. S. KARNS, D. R. WOLFGANG, E. HOVINGH, B. M. JAYARAO, C. P. VAN TASSELL, and Y. H. SCHUKKEN. 2008. Environmental sampling to predict fecal prevalence of *Salmonella* in an intensively monitored dairy herd. *Journal of Food Protection* **71**: 1967–1973.

155. VILLANI, F., M. APONTE, G. BLAIOTTA, G. MAURIELLO, O. PEPE, and G. MOSCHETTI. 2001. Detection and characterization of a bacteriocin, garviecin L1-5, produced by *Lactococcus garvieae* isolated from cow's milk. *Journal of Applied Microbiology* **90**: 430–439.

156. VISSERS, M. M. M., F. DRIEHUIS, M. C. TE GRIFFEL, P. DE JONG, and J. M. G. LANKVELD. 2007. Short communication: quantification of the transmission of microorganisms to milk via dirt attached to the exterior of teats. *Journal of Dairy Science* **90**: 3579–3582.

157. VOUGHT, K. J., and S. R. TATINI. 1998. *Salmonella* Enteritidis contamination of ice cream associated with a 1994 multistate outbreak. *Journal of Food Protection* **61**: 5–10.

158. WAAK, E., W. THAM, and M. L. DANIELSSON-THAM. 2002. Prevalence of *Listeria monocytogenes* strains isolated from raw whole milk in farm bulk tanks and dairy plants receiving tanks. *Applied and Environmental Microbiology* **68**: 3366–3370.

159. WHEELER, T. T., A. J. HODGKINSON, C. G. PROSSER, and S. R. DAVIS. 2007. Immune components of colostrum and milk – A historical perspective. *Journal of Mammary Gland Biology and Neoplasia* **12**: 237–247.

160. WHITE, D. G., R. J. HARMON, J. E. MATOS, and B. E. LANGLOIS. 1989. Isolation and identification of coagulase-negative *Staphylococcus* species from bovine body sites and streak canals of nulliparous heifers. *Journal of Dairy Science* **72**: 1886–1892.

161. WILSON, J. B., R. C. CLARKE, S. A. RENWICK, K. RAHN, R. P. JOHNSON, M. A. KARMALI, H. LIOR, D. ALVES, C. L. GYLES, K. S. SANDHU, S. A. MCEWEN, and J. S. SPIKA. 1996. Vero cytotoxigenic *Escherichia coli* infection in dairy farm families. *Journal of Infectious Diseases* **174**: 1021–1027.

162. WITHERS, H. 2008. It all begins down on the farm. Presentation at Meat Industry Workshop, 21 October 2008. AgResearch Ruakura, Hamilton, New Zealand. [Online.] www.paracotechnologies.com/.../04-it-all-begins-down-on-the-farm.pdf

3

Key requirements for milk quality and safety: a processor's perspective

K. Burgess, Dairy Crest, UK

Abstract: This chapter reviews the various requirements of milk processors for their supplies of raw milk. It also takes a historical perspective, looking at previous arrangements, how they have changed to the present day, and how they may change in the future. From a present day perspective, the milk processors' concerns include food safety, food quality, environment and sustainability, technical innovation, the ability to supply 'niche' milks and the ability to drive cost efficiency. The mechanism for the delivery of these is then reviewed in the context of milk supply contracts, and there follows a consideration of what processors may be looking for in addition to this base level. Farm assurance programmes are then suggested as an appropriate vehicle for capturing the processor's requirements and as a basis for on-going improvement initiatives, including some possible future trends.

Key words: milk quality, milk supply contract, milk safety, dairy farm assurance.

3.1   Introduction

The bottom line for milk processors is ensuring a supply of good quality raw milk that can be used as the basis for manufacturing a wide range of innovative, high quality and safe dairy products for the customers and consumers they serve. It is also a general truism that once raw milk is defective, it is most unlikely that it can be improved during processing, and that defects will more often than not become more pronounced.

Additionally, the requirements for milk quality and safety change in line with the ongoing changes that markets face as a result of the relentless shifts in

political, economic, social and technological factors in the business environment. This means that in any product supply situation, the basic standards of today get inextricably linked with the additional expectations of today's customers to become the new standards of tomorrow.

The key requirements for milk quality and safety today, from a processor's perspective, therefore reflect what has become more important to the industry's customers and consumers over the past few years.

As an example and a starting point, the issue of key requirements for milk quality was addressed some 25 years ago (Gordon, 1984). The key issues Gordon identified would now be considered as the most basic chemical, microbiological and physical standards concerning levels of:

- Appearance, smell and taste
- Temperature
- Solids not fat
- Freezing point depression
- Antibiotics
- Acidity
- Sediment
- Resazurin
- Total bacterial count
- Thermoduric count.

From this list we can see some clear direction of changes that have happened in the ensuing 25 years, where either:

- the requirement is no longer on the processor's radar because it is now well managed at farm level (e.g., sediment), or
- the requirement is now contracted on a performance basis, as opposed to a minimum standard (e.g., fat, protein levels, bacterial counts), or
- new requirements are needed for the modern business environment (e.g., somatic cell count).

3.2   Key elements of the processor's perspective

The most important elements of raw milk quality from the modern processor's perspective can be grouped into the following categories:

- Food safety
- Food quality
- Environment and sustainability
- Technical innovation
- Ability to supply 'niche' milks
- Ability to drive cost efficiency.

The key requirements for raw milk quality and safety have therefore moved on considerably from the situation 25 years ago. Of course, the standards for

consumer food safety and food quality still form the basis of these requirements, but the nature of modern food safety and food quality has moved forward on several fronts.

In the first place, many of those chemical and microbiological standards have become more stringent as analytical methods have become more sensitive and operating practices at farm level have improved. Also, many of the micro-biological and chemical milk quality issues of 2009 were not even suspected in the 1980s, so this is a second level of change. The changes up to this stage relate to the basic concepts of food safety and food quality, which always form the basis for meeting end customer and consumer requirements.

The next level of expectation relates to the changes in the business and consumer environments of the past few years. Here we can include the massive increase in the importance of sustainability and the environment in current business and consumer priorities.

At yet another level, there are aspects of milk quality and safety from the processor's perspective that increasingly relate to the processor's ability to compete commercially. Here, the processor has an expectation of his or her supplier to have competence in driving cost efficiency, expertise in the area of innovation, and an infrastructure that gives the possibility of producing 'niche' milks with specific nutritional and physical characteristics (e.g., omega 3).

We can see from these enhanced levels of requirements that the concept of the word 'quality' from the processor's perspective has widened considerably since the view put forward by Gordon those 25 years ago. However, as set out earlier, the issues of food safety and quality will always be the bedrock of the processor's concern, so it is appropriate to start with a consideration of these first in the context of a basic milk supply contract.

3.3   Basic requirements: essentials of the contract to supply milk

The purchasing of raw milk by a processor is no different from any other commercial purchasing transaction in that it is usually based on a formal documented contract. The contract with the processor may be agreed between individual milk producers, between groups of producers, or with larger organizations such as cooperatives. Whichever the case, a basic milk contract will contain reference to the following:

- Basic price per litre
- Compositional quality payments for fat and protein
- Bacterial count, in terms of both a target and penalties when the target is not achieved
- Somatic cell count, again in terms of a target and penalties when the target is not achieved
- Collection arrangements (frequency of collection from the farm, and associated payment)

- Seasonality bonuses and penalties
- Quality bonus.

While the contract for milk supply will include details of all these parameters, there will also be in place a monitoring programme both to ensure that the required standards are being met, and as a basis for the payment of bonuses and penalties. The latter are an important basis for the processor to work with producers to provide the appropriate motivation to drive milk quality parameters, ideally linked to the processor's manufacturing requirements.

We can now review the basic aspects of the milk contract in more detail.

3.3.1   Compositional quality payments

While most milk producers have been paid for milk on the basis of delivered volume and composition for some years now, there is an increasing tendency for the basis of such payment to be tied much more closely to the specific products being produced by the processor. An illustration of this can be given by the difference in approach of a UK group between milk used for market milk production and milk used for cheese production (DFOB, 2008).

For market milk production there is currently no benefit in a raised protein content, so there is no premium paid for protein levels above the minimum standard of 2.9%. This situation may be different in countries where the protein standardization of market milk is permitted. Also, in the case of market milk, there is usually only a small premium paid for elevated fat levels, so that over 75% of the payment is based on volume alone.

While market milk is usually sold on the basis of volume alone, this is not the case with cheese manufacturers where the yield of the cheese produced depends primarily on the protein and fat content of the milk. In this case the contract would be much more focused on driving the payment bonus along the lines of encouraging higher levels of protein and fat to the extent that only some 40% of the overall payment would be related to volume alone.

It can be seen from this example that the processor can work with producers to maximize the benefit to both parties, in terms of maximizing the return to producers for supplying milk of the optimum composition to the processor and the products being manufactured.

The other aspect of milk compositional payment that needs to be considered is any penalty to be invoked if the total solids content of the milk is too low as a result of adulteration with water. The freezing point depression is the well-established method for monitoring this, with a freezing point of $-0.512°C$ (previously $-0.530°H$) and below commonly accepted as the standard for unadulterated milk.

3.3.2   Bacterial standards

Most countries have a legal standard for the maximum number of bacteria in raw milk, which is normally set at 100,000 cfu/ml. However, freshly drawn milk

from a healthy cow is very low in bacterial numbers and these only increase to levels near 100,000 cfu/ml or more as a result of poor milking practice, inadequate cleaning of farm equipment, poor cooling of the milk or poor herd health. With good hygienic practice at farm level it is therefore possible to produce milk with a bacterial count of some 10,000 cfu/ml or less and most milk supply contracts will include provision for encouraging lower bacterial counts than the legal minimum.

Bacterial counts are measured in different ways depending on the mechanics of the laboratory testing process. In smaller operations, the traditional poured plate incubation methodology is used, while in larger, mechanized laboratory testing operations the automated Bactoscan (staining live and dead bacteria followed by microscopic detection) is the preferred method.

With bacterial counts, the processor will usually agree a well-achievable good practice level as the standard, e.g. a Bactoscan count of 50,000 (FOSS, 2009), and counts below this will not receive a premium. However, on a rising scale, counts above this will receive increasingly higher levels of penalties.

This payment approach for hygienic quality is deliberately positioned by the processor to provide the producer with the greatest motivation to supply raw milk of good keeping quality and low in pathogens. While in the example given there was no incentive for even better hygienic quality than the base level, there are actually ways to motivate even higher hygiene standards:

- Making hygiene one of a number of key performance indicators, which if consistently achieved over a period will lead to a bonus payment.
- Developing added value contracts for the supply of milk to premium retailers where a higher milk price can be paid for even higher consistent hygiene standards.

3.3.3   Somatic cell counts

Somatic cell counts in milk are of interest to the processor for a number of reasons. Somatic cells (mainly blood cells that fight infection) are present in milk in significant numbers only in order to combat bacterial infections of which mastitis, an infection of the udder, is the most common cause of concern.

The presence of significant numbers of somatic cells, and therefore evidence of mastitis in the herd, is of concern to the processor for two reasons:

1. The presence of mastitis is an indicator that herd health is not as good as it should be, and therefore a matter of concern when it comes to the health and well-being of the animals.
2. Cows with mastitis give milk that is lower in quality and wholesomeness. This can be manifested in one or more of the following ways:
   - Enzymic activity of the infecting bacteria causing degradation of the milk fat and protein
   - Presence of pathogens (infecting bacteria)
   - Reduced yield of some manufactured products (e.g., cheese)

- Flavour defects (bitterness, rancidity).

For these reasons, legal standards for somatic cell counts are set in most countries, but these can vary widely between 250,000 and 750,000 per ml. The lower level is a common standard in many countries now because it is accepted that somatic cell counts above 250,000 per ml give a strong indication that there is mastitis in the herd, even at a sub-clinical level.

In terms of milk contracts, the processor's approach to dealing with somatic cell counts is similar to that seen with bacterial standards, i.e. the expectation of a good practice level and the application of a rising scale of financial penalties when these good practice levels are not met.

3.3.4   Antibiotic arrangements

The issue of mastitis in dairy cattle has already been raised in the context of somatic cell counts. Mastitis is most commonly managed through treatment with antibiotics, usually the beta lactam antibiotics of the penicillin family.

Residues of antibiotics in raw milk are a serious concern to the processor for a number of reasons:

- At a basic level, there are legal limits for antibiotic residues in milk which must be met.
- An increasing proportion of the population is allergic to beta lactam antibiotics. This may be a low public health risk but it is high on the consumer agenda and therefore very relevant to the processor.
- Many dairy products depend for their quality characteristics on the successful growth and development of lactic acid starter cultures. The presence of significant levels of antibiotic residues can result in partial or even complete inactivation of starter culture activity, leading to inadequate fermentation and associated texture and flavour defects.
- There is an increasing concern in the scientific community and the public arena about the increasing resistance of several human pathogens to treatment with antibiotics, and the possible links with the use of antibiotics in food animals. Again this is not a proven risk but once more it is an issue high on the consumer agenda and thus also on the processor's.

For these reasons control of antibiotic residues in raw milk is of the highest priority for the processor, and that is why contracts for raw milk supply usually include measures for very draconian financial penalties where antibiotic residues exceed legal limits. Consistent breach of antibiotic standards by a producer will inevitably have calamitous financial implications for them, because after a few failures, insurance protection will be withdrawn, leaving the producer potentially liable for entire silos of milk if their supply can be proved to be the source of any contamination.

In spite of the very large financial downside to the producer of exceeding legal limits for antibiotic residues, between 0.1% and 0.5% of milk tankers still test positive for antibiotics even in developed countries (Tsaknis and Lalas, 2004).

The reasons for this are threefold. In the first place, the technical communication between antibiotic suppliers, vets, farmers and processors is not as good as it should be. For example, there is a general understanding across the dairy industry supply chain that milk from cows treated with antibiotics needs to be held for a set time until the antibiotic has worked its way out of the cow's metabolic system. This withholding time is usually defined by the antibiotic supplier and these recommended times are generally respected by vets and farmers. However, it is not unusual for the antibiotic supplier to be using laboratory test methods specific for their proprietary antibiotic, while the raw milk testing laboratory and the processor's intake laboratory are using test methods which are more generic. The latter are designed to detect a much broader range of antibiotics than a specific supplier's, and hence there can be discrepancies. Inevitably, this means that producers can follow antibiotic supplier's guidelines and still sometimes fail the test undertaken by raw milk testing laboratories.

Another reason for ongoing antibiotic failures relates to the natural variation that is seen between individual cattle. Antibiotic supplier recommendations are obviously based on average observations and equally obviously there is a natural distribution of differences in withholding times between individual cows. The precautionary principle would indicate that the most prudent choice would be to select the longest withholding time within the natural range of variation. However, there are two factors that compromise this approach:

- The suppliers of antibiotic preparations will naturally want to give an optimistic view of withholding times for competitive reasons.
- The farmer will want to minimize the withholding time to reduce the amount of milk he will have to discard.

The third main reason for ongoing antibiotic failures is the periodic lack of adherence to good farming practice with respect to antibiotic control. There are several good practices which help ensure that antibiotic-contaminated milk will not enter the general milk supply, and it is increasingly a priority of the processor to develop an improvement plan with their supply base to make sure that these practices are adhered to. Some of these practices relate to good farming practice in general and these will be dealt with later. However, the specific practices for antibiotic control include:

- Correct operation and maintenance of milking equipment to reduce the likelihood of mastitis in the first place.
- Use of qualified vets to administer antibiotics with well-established withholding times.
- Correct administration of antibiotics and reliable recording of this.
- Obvious identification of treated animals.
- Antibiotic-treated cows milked last, with this milk discarded and the equipment well cleaned before reuse.
- Effective communication of antibiotic treatments to all farm personnel.

Many processors now also strongly encourage producers to test for antibiotics on farms when there is any uncertainty about antibiotic residues still being present in raw milk. Increasingly, processors help fund such on-farm testing to encourage the positive release of any suspect milk prior to collection.

3.3.5   Sensory quality

Most contracts for raw milk supply refer to the need for the absence of flavour defects. Milk naturally has a fairly bland taste, with a slight degree of sweetness arising from the lactose content. However, there are a number of sources of flavour defects in ex-farm milk and there needs to be a process in place that ensures that these do not get transmitted into finished products. Such flavour defects can arise from:

- Chlorophenol taint: from the reaction between phenolic chemicals (some farm disinfectants and preservatives) with free chlorine.
- Feed taints: strong feed odours from feed (silage, molasses) or from wild flora in pasture (onion, garlic).
- Oxidized: cardboard flavour from oxidized milk fat.
- Rancid: fat breakdown by lipases to produce free fatty acids.

All of these potential flavour defects are preventable with good dialogue between producer and processor. However, for the sake of due diligence, a processor should also have in place a sensory screening programme, with appropriately trained tasters, who can identify these potentially important quality defects before they can impact on product quality.

3.3.6   Collection arrangements and seasonality

While the basics of milk quality and safety (composition, bacterial count, somatic cells, antibiotics) are fundamental to the processor's requirements for a raw milk supply, the way in which milk is delivered to the processor is also key for economic as well as quality reasons. For this reason milk supply contracts usually include terms related to how often the milk can be collected from the producer, and what milk will be available at different times of the year. From a hygienic quality perspective, collection of milk every other day from the farm is sufficiently frequent and there is obviously an economic saving in transport costs if milk can be collected at this frequency rather than daily.

   The other key priority for the processor with regard to milk supply is how the monthly supply volume ties in with the processor's production plan. This requirement differs from processor to processor as those producing short-life products probably need an even milk supply volume from month to month, while those producing longer shelf-life products (UHT milk, butter, cheese) can usually accommodate a more variable supply pattern.

   Once again these processor requirements can be built into the basic milk supply contract using appropriate financial bonuses and penalties.

3.3.7   Quality bonus

As we conclude the discussion on the key day-to-day elements of a raw milk contract which sets out the processor's requirements for food safety and quality, it is important to remember the motivational importance of encouraging milk producers to be continually improving safety and quality standards. One approach to this is to reward producers with a bonus payment that reflects consistent performance in terms of achieving standards for somatic cell content, bacterial count and the absence of antibiotic failures.

3.4   Beyond the basic milk contract: additional requirements

The raw milk food safety and quality issues discussed so far are the basics of the day-to-day supply of milk to the processor. While they are essential for the regular operation of the milk supply chain, there are also other considerations which the processor must include within the overall scope of their milk safety and quality arrangements. These considerations fall into two categories:

- The drive for continuous quality improvement
- The ability of the producer–processor relationship to deal with significant food safety and quality issues that may enter the consumer arena.

The drive for continuous quality improvement relates mainly to hygienic milk quality and to the increasing awareness of consumers about animal welfare standards on farms, and provenance. The ability to deal with significant industry safety and quality issues depends on developing a sound knowledge base in the key areas of interest. These concepts are now discussed further.

3.4.1   Continuous quality improvement

Of course, quality improvement processes could be applied across all areas of milk safety and quality parameters already mentioned, and individual processors will prioritize the areas on which they want to focus for their own particular situations.

For the purposes of this discussion we will focus on two examples, both related to aspects of milk hygiene. These relate to approaches that could be taken to improve bacterial counts and somatic cell counts.

Bacterial counts

The bacterial count has already been mentioned as a very important indicator of milk quality, but it is a very blunt one as it does nothing to distinguish between the different types of bacteria in raw milk and their relative impacts on milk quality and safety. Of these different bacterial groups, probably psychrotrophs and thermoduric bacteria are the ones that can have the most important adverse impact on milk quality. For example, in hygienically produced milk, less than 10% of the overall bacterial count will be psychrotrophs. However, a less

hygienically produced milk could have up to 75% psychrotrophs and yet still have a similar overall bacterial count (te Giffel, 2003).

What is needed, therefore, are more discriminatory tests that can provide trend information on the bacterial groups most related to quality. While a number of these are coming into use around the world, two examples from Cornell University warrant attention (Cornell University, 2007).

The preliminary incubation count (PIC) is a means of identifying numbers of bacteria in raw milk that are capable of growing at cooler temperatures. Milk samples are held at 12.8°C for 18 hours and then subjected to a standard bacterial count test. Milk produced hygienically (see above) should not show a PIC significantly greater than the standard plate count for the raw milk. However, less hygienically produced milk may have a PIC of some 3–4 times the original plate count (Cornell University, 2007). As an improvement tool, it is therefore possible to set targets in terms of the PIC as a proportion of the standard plate count, as a means of driving improvements in farm cleaning, disinfection and milk handling procedures.

On the other hand, the laboratory pasteurization count (LPC) gives an indication of the presence of thermoduric bacteria in milk. These are bacteria that can survive the pasteurization process and are important determinants of finished milk product quality, particularly when the distribution chill chain is not absolutely in control. Again, hygienically produced milk which is stored at the correct temperature should not contain significant numbers of thermoduric bacteria. A simple test for these sorts of bacteria is to laboratory pasteurize raw milk at 62.8°C for 30 minutes, and then carry out a standard plate count on the heat-treated milk. Hygienically produced milk should contain no more than a small number of tens of these organisms per ml. Numbers higher than this are a strong indicator of poor udder hygiene and/or poor control of temperature on the farm. Again this measure can be a useful indicator in the programme to improve the bacterial quality of ex-farm milk.

Somatic cell counts
While specific types of bacterial counts (i.e. numbers) can be used as a basis for quality improvement, an alternative approach can be the use of subjective scores as a basis for improvement. Several examples of this have been seen in the farming sector for decades now, for example with body condition scoring and lameness scoring, and they have been very effective in driving improvement in these important areas.

This approach has also been reported with another important indicator of raw milk quality and cattle health, that is, the somatic cell count mentioned earlier (Reneau *et al.*, 2003). In this case a means of subjective scoring of cow hygiene was established and this was related to somatic cell counts. Reneau *et al.* (2003) reviewed a number of subjective cow hygiene scores related to the different parts of a cow's body, and examined their relation to somatic cell counts. A statistical analysis of the results showed that it was only necessary to score the hygienic status of the cow's lower rear legs and udders to establish a highly

significant relationship with somatic cell count. A scoring scale of 1 to 5 was used (1 equating to absolutely clean, 5 relating to very dirty) and it was established that for each unit change in cow hygiene score, a reduction in herd somatic cell count of 40,000–50,000 per ml could be expected.

From these considerations of quality improvement in bacterial counts and somatic cell counts, it is clear that both objective and subjective bases for improvement can deliver improvements. This concept of quality improvement, as a cooperative process between processor and producer, should always be a prime consideration for the processor in terms of raw milk supply.

3.4.2   Industry safety issues

Industry safety issues can arise from both microbiological and chemical causes, and it is a key requirement for the processor that there are producer and processor agreed arrangements in place to manage the risks and deal with such issues when they have a raised public profile.

Microbiological issues
In the earlier discussion on raw milk hygiene it was recognized that there is an inevitability to raw milk suffering at least some contamination during the milking operation and subsequent storage. While the hygiene improvement measures mentioned earlier will contribute to reducing this, it is still important to be aware of the key microbiological issues facing the industry in the widest sense.

Pathogens
Milk is an ideal growth medium for a wide range of pathogens and these have recently been well summarized (Jooste and Anelich, 2008; O'Mahoney *et al.*, 2009). However, because the dairy industry is so well developed, there are well-established methodologies for controlling them, which typically include:

- Chilled storage
- Pasteurization process
- Cleaning and sanitation of equipment
- Measures for avoidance of cross-contamination.

These measures are appropriate and sufficient when it is established that regulatory pasteurization conditions are known to destroy pathogens of concern, and that cleaning and disinfection measures have a similar impact.

In spite of this, there are always potential microbial pathogens, which should be kept under regular surveillance in view of their relatively higher likelihood of becoming public health issues. A good example of this is *Mycobacterium avium* subsp. *paratuberculosis* (MAP).

MAP
As indicated above, all of the known and confirmed pathogens in milk are effectively destroyed by the pasteurization process. However, from time to time

new science exposes the possibility of emerging micro-organisms which may not be killed by this process.

When *Listeria* became a serious public health issue in the 1980s, there was significant controversy over whether the organism was killed by commercial pasteurization. Once all the science had been correctly interpreted, it was eventually confirmed that conventional pasteurization was indeed sufficient to kill *Listeria* species.

A key current issue that falls into this category is that of MAP. The issue has actually been around for some time now, and goes back to the fact that MAP was found to be the causative agent of Johne's disease in cattle over 100 years ago (Hillerton, 2003). This disease affects the intestine of the animal, causing inflammation and resulting in symptoms of diarrhoea and chronic weight loss.

Throughout most of the 1900s, Johne's disease was viewed as an animal disease that was managed on the basis of cost–benefit parameters, i.e. the measures taken to prevent the disease were weighed against the cost–benefit of their impact. In the 1990s however, concern was raised over the possibility that MAP may also be the cause of Crohn's disease in humans, a disease with many symptoms similar to those of Johne's disease in cattle.

While no scientific link has been proven between MAP and Crohn's disease, the situation in the late 1990s and early 2000s was accentuated by reports from Queen's University, Belfast (Grant *et al.*, 2002) that MAP could survive the normal pasteurization process. Subsequently, research groups in the UK (Grant *et al.*, 2005) and the Netherlands (Gallmann and Eberhard, 2004) reported pasteurization bacterial log reductions of between 4 and 5.2, more than sufficient to provide for pasteurized milk safety. Also, Gallmann and Eberhard (2004) reported that the Queen's University work contained significant flaws and should be disregarded.

While the link between MAP and Crohn's has no scientific basis, and the overwhelming balance of evidence confirms that MAP is destroyed by conventional pasteurization, it is still good practice based on the precautionary principle to minimize the incidence of MAP in raw milk. This is an area where the processor would expect to be working with supplying producers to establish best agricultural practices for reducing MAP counts in raw milk.

The main source of any MAP in raw milk is through faecal contamination (Heggum, 2001). Reduction in the level of raw milk contamination by MAP should therefore focus on:

- Reducing the incidence of Johne's disease at the herd level, through improved herd health and the culling of infected cattle.
- Reducing the level of faecal contamination during milking by adopting best hygienic practice to ensure that the udders are cleaned of all faecal matter and bacterial contamination prior to the milking process.

With these measures in place at farm level, the responsibility is thereafter fully with the processor to ensure proper control of the well-established pasteurization

process that will destroy any remaining MAP and render the milk safe for the consumer.

Chemical issues

As was mentioned with microbiological issues, the industry is well developed and would not be expected to have significant issues with chemical contaminants. However, recent examples of issues in Europe include lead-contaminated cattle feed in 1989, the incidence of BSE in the UK in the 1980s and 1990s, and the incidence of PCBs in Belgium in the 1990s. Issues of chemical contamination from milk are therefore still an agenda item for the processor's crisis management checklist, and it is appropriate to review three examples of this.

BSE

A major issue affecting the UK cattle industries in the 1980s and 1990s was an epidemic of BSE, now colloquially known as 'mad cow disease'. The origins of this outbreak are now agreed as having arisen from changes in the processing technology of animal feed that allowed the causative agent to survive. Previously, the causative agent (prions) had been destroyed by the heat treatment used. The risk of feeding animal products back to their own species (meat and bonemeal) was also recognized at the time and has subsequently been legislated for.

Prions are the infective particles that can transmit the disease between cells and between animals. They are protein based and when they enter brain cells they have the ability to convert normal cell protein to the infected form. As more of the infected form of the cell develops, there is an increasing blockage of the normal brain cells, which eventually results in cells dying and forming holes in the brain. Under electron microscopy the brain at this stage appears like a sponge, and this type of disease is not surprisingly called a spongiform encephalopathy.

While BSE was an obvious disease and a financial issue for the beef and dairy cattle sectors, in the early stages there was no link to human disease. This situation changed with the recognition of a new variant of a well-known but rare disease known as CJD. While there was no direct evidence of a link between BSE in cattle and CJD in humans, the epidemiological evidence of a link was overwhelming, and a number of measures were introduced first in the UK and then more widely across the EU to ensure potentially contaminated material was removed from the food chain.

From a milk perspective, several studies have investigated the possibility of a link between BSE infection in cattle and the presence of the infective agent in milk. These have been extensively reviewed and it has been consistently concluded that there is no such link (TAFS, 2007). These findings are shared by the USDA (USDA, 2005).

However, in spite of the absence of any scientific link between milk and BSE, there were many examples in the 1990s of the dairy industry being significantly

commercially disadvantaged because of the association between dairy cattle and BSE. This was the result of two factors:

- The processing sector of the dairy industry had very little involvement in the cattle feed production industry and the changes going on within it. Processors were therefore unaware of any potential risks.
- While the issue of BSE was in the scientific domain in the 1980s and early 1990s, it was not for several years that processors realized the extent of the possible association with milk.

Lessons have been learned from the BSE outbreak in the UK, namely that processors need to be aware in detail of animal feed processing technology, that government regulations cannot be totally relied on for control, and that there is a need for a risk management awareness of everything that affects the cattle industry and not just milk itself.

Environmental contaminants

A number of potential environmental contaminants of milk have been known for some time now:

- Heavy metals
- Pesticides
- Dioxins
- PCBs.

It is important for the processor to have knowledge of trends and changes in these entities for two important reasons:

- In many parts of the world legislation is becoming progressively stricter in the control of such contaminants.
- Scientific advances may show additional risks from these chemicals.

The other key factor relating to these types of contaminants in the dairy supply chain is again a detailed knowledge of the safety of the feed supply to the dairy cattle supplying the processor's milk, whether it is grass or bought-in feed.

Mycotoxins

The issue of mycotoxins is another example of the need of the processor to be more closely involved in the supply chain of animal feed to dairy cattle. Mycotoxins are metabolites of various types of fungi, e.g. *Aspergillus flavus*, and pose a risk to human health since some of them are carcinogenic and/or neurotoxic. Their entry point into the dairy supply chain is in some of the various feeds used to supplement grass in the feeding of dairy cows, e.g. cereals and some nuts.

   The most important potential mycotoxin in milk is aflatoxin M1, and the extent of concern about this contaminant is evidenced by the fact that its levels are legislated for in several countries, and that there is also a Codex maximum limit for aflatoxin M1, set at $0.5\,\mu$g/kg.

The issue of mycotoxins such as aflatoxin M1 in milk is yet another example of the need for the processor to take a preventive approach when it comes to safety issues in cattle feed. Mycotoxins cannot be eliminated from feed once it has become contaminated, so preventive measures such as:

- prevention of physical crop damage by insects
- prevention of physical damage at harvest
- crop cleaning to remove soil
- keeping crops clean and dry

are key to avoiding contamination of feed crops in the first place. At the same time an important part of the mycotoxin control process is a continuous monitoring programme. It is vital that the processor is involved in these preventive and monitoring programmes.

3.5   A vehicle for future quality and safety improvement: farm assurance programmes

While there will inevitably be specific issues for agreement between processor and supplier on milk quality and safety issues (e.g. specific standards for bacteria, somatic cell counts, etc.), a vehicle is also required for all of the generic improvements that need to be put in place year on year.

Until the last decade or so, quality assurance (QA) systems were mainly to be found towards the consumer end of the supply chain, particularly with retailers and manufacturers. For several reasons, the imperatives to include farming operations within the QA context have moved forward significantly over recent years, and these can be set out as follows:

- The need for much greater focus on control of the feed supply chain as set out in Section 3.4 above
- A basis for establishing and driving forward good hygienic practices
- The need to ensure that current and potential future industry risks are incorporated into farm management practices
- A basis for driving forward the standards of animal welfare that are increasingly becoming an expectation of consumers.

A number of such farm assurance programmes are now in place in many countries over the world, and from a dairy perspective the International Dairy Federation (IDF) is probably the best source of information (IDF, 2004).

3.5.1   Good agricultural practices

The IDF Guide sets out the required Good Agricultural Practices (GAP) in the following areas:

- Animal health
- Milking hygiene

- Animal feeding and water
- Animal welfare
- Environment.

In addition to these general areas of Good Agricultural Practice, the IDF Guide also sets out requirements for traceability and record keeping in the following key areas:

- Use of agricultural chemicals and veterinary medicines
- Purchase and use of animal feed
- The unique identification of individual animals.

For the purposes of this discussion, it is important to understand the meaning of traceability in the dairy sector. The concept of traceability usually means the ability to trace back all of the ingredients of a particular product, together with the ability to track forward where all of the batches of a particular product have been distributed. However, as mentioned above (IDF, 2004), in the context of milk supply from a herd of dairy cattle the concept of traceability is actually wider than this (Jooste and Anelich, 2008) and in addition includes the retention of accurate records of:

- Livestock treatments (medications by animal)
- Soil treatments (fertilizers, pesticides used)
- Purchased animal feed (description, list of ingredients, batch number, supplier)
- Feeding regime
- Cattle registration (of cows bought and sold).

A key part of the processor's concern for traceability is the retention of samples at different stages in the supply chain to enable a trace back in the event of a subsequent problem. The key points for sample retention are:

- Farm milk tank
- Road tanker
- Work in progress (partly processed product)
- Finished product.

With a combination of the knowledge of the sources of inputs, and retained samples at key steps in the supply and processing chain, the processor is in a good position to access all the information necessary to manage any crisis management situation. These controls are made more robust by the fact that individual farms wishing to be accredited to such assurance schemes have to be audited on a regular basis by third-party accredited auditors.

3.5.2   Animal welfare

Another feature of farm assurance schemes in the dairy sector is their focus on the animal welfare of dairy cattle. This is of increasing concern to consumers, and therefore also to processors. The IDF 2004 Guide was updated in 2008 to

reflect this (IDF, 2008). This takes as its basis the now widely accepted freedoms expected in ethical farming: freedom from thirst, hunger and malnutrition; freedom from discomfort; freedom from pain, injury and disease; freedom from fear and distress; and freedom to engage in normal patterns of animal behaviour. In fact the IDF Guide presents a more practical interpretation of these requirements, setting out that animals should:

- Live in reasonable harmony with their environment
- Have adequate fulfilment of their physical, health and behavioural needs
- Not be subjected to unnecessary or unreasonable pain or distress.

It then goes on to identify the five key action areas to be considered in delivering this, and gives details of what is required under each:

- Stockmanship
- Feed and water
- Physical environment
- Husbandry practices
- Health management.

3.5.3   General

The concept of dairy farm assurance schemes can therefore be seen as a very appropriate vehicle for the processor to agree with producers any changes in farm management practices that need to incorporate new scientific knowledge or new consumer expectations. The IDF Guides to good dairy farming practice and good animal welfare represent very good bases for such schemes, since they incorporate concepts that have been learned from recent food safety issues and areas of growing consumer concern:

- Integrity and traceability of animal feed and milk
- Animal welfare conditions
- Health and disease avoidance in dairy cattle
- Minimization of the environmental impact of dairy farming.

3.6 Future trends

The two key drivers for technical change over recent times have been information technology and biotechnology. In some cases the two are very much related, for example establishing the genome of dairy cattle using the information processing power of computer technology. However, in the case of milk products, biotechnology has been the most important of these drivers.

Biotechnology in the context of milk supply can be seen in three possible application areas:

- Use of GM to produce cattle feed
- Use of GM to improve cattle genetics

- Use of GM to enable cattle to produce added-value constituents in milk.

The first of these, using GM to produce cattle feed, is already a commercial reality. Most of the bought-in feed in the UK is already GM based, and the same trend is seen worldwide. This is not a particular issue for the processor, since several studies have demonstrated that any GM material ingested by the cow is broken down by the cow's metabolism and cannot be detected in milk.

The use of GM to improve cattle genetics is more contentious because of the issue of consumer acceptability. In the first place consumer acceptance of GM food is still an important issue in many countries, and secondly milk is seen as such an important food for infants and young people that the likelihood of consumer acceptance of GM cattle for regular milk supply is highly unlikely.

The third potential application of GM to dairy cattle, to enable the production of added-value pharmaceutical-type constituents, is a more likely application of GM in the industry going forward. However, this is likely to be on a contract-by-contract basis rather than a generic change because of the costs involved.

While consumer concern over the use of GM to modify regular milk composition is likely to be a significant barrier in the short to medium term, at some stage in the future GM technology will probably become an accepted approach. In the meantime, processors will still have an interest in working with producers to develop milks with a particular composition that is suited to products they manufacture.

Mention has already been made of composition-based payment systems to encourage the production of milk, for example an ideal ratio of fat to protein for cheesemaking. With developments in the knowledge base of how feed inputs affect the relative production of individual milk components, there is an increased likelihood that processors and producers will together examine new feeding regimes to see if they can generate milks with more appropriate fat and protein contents for specific products.

However, the processor also needs to consider whether it is more appropriate to manipulate raw milk composition through feeding, or to accomplish the same result through the use of processing technology. With the ability to control milk fat composition through separation and standardization processes, and the ability to modify the protein content of milk via ultrafiltration, the processor is probably in a better position than the producer to modify these milk compositional factors.

This will be a fundamental decision for processors going forward: selecting the most cost-effective means of achieving the balance of fat and protein they want in milk for the products they produce. In the author's experience, most value can be added for the processor when the means of compositional manipulation are within their own control.

The other future trend of concern to the processor is the issue of sustainability. Mention has been made earlier of environmental issues, and for the dairy supply sector this first means ensuring that dairy operations do not pollute the environment. At the next level, dairy operations need to minimize the resources they need in terms of energy and raw materials consumed. At a third

level, the milk supply sector must consider the impact of dairy farming on global warming. While the first two phases of environmental improvement for the dairy sector were effectively self-funding (reduced product losses, reduced energy usage), addressing the issue of sustainability represents a step change.

The next stage in developing sustainability for the milk supply sector is addressing the basic fact that dairy cattle produce a substantial proportion of the world's greenhouse gases. Domestic livestock produce around 26% of all methane emissions (Boland, 2003) and cattle account for most of this. Several research programmes are currently in place worldwide to address this and it is likely that scenarios will soon be developed that modify the operation of the cow's rumen to significantly reduce the amount of methane produced. One such approach (Dairy UK, 2008) is based on optimizing forage fibre to help animals chew more and convert their feed into energy more efficiently. Results to date indicate that a reduction of more than 20% can be achieved, alongside a slight increase in milk yield.

The concept of sustainability in the milk supply sector is likely to be a key driver going forward.

3.7   Sources of further information and advice

The most comprehensive source of information on raw milk quality and safety is the International Dairy Federation (IDF). This organization has been in existence since 1903 and its member countries account for around 75% of all the milk produced globally. The objectives of the IDF are:

- Developing scientific knowledge
- Exchanging information
- Addressing global development
- Facilitating networking within and outside the sector.

The IDF website (www.fil-idf.org) is available to all, but some publications may need specific user names and passwords. For member countries, these can be easily obtained from the individual country's National Committee of the IDF.

The Society of Dairy Technology (www.sdt.org/default.htm) is another good source of dairy information, both through its quarterly journal, the *International Journal of Dairy Technology*, and through a series of Dairy Science and Technology related texts published during 2008 and 2009. A related publication, the *Australian Journal of Dairy Technology* is also an excellent source of relevant papers.

There are many Internet websites which purport to give information on the dairy science and technology of raw milk. However, many of these are not validated and they should therefore be treated mostly as a source of interest for further investigation, rather than as *bone fide* sources of information. The exceptions to this are some of the university websites, particularly those of the Universities of Guelph, Cornell and Minnesota.

3.8   References

BOLAND, M. (2003) Influences on raw milk quality. In: Smit, G. (ed.) *Dairy Processing Improving Quality*, pp. 42–80, Woodhead Publishing, Cambridge.

CORNELL UNIVERSITY (2007) *Importance of Raw Milk Quality on Processed Dairy Products*. Dairy Foods Science Notes, pp. 1–2.

DAIRY FARMERS OF BRITAIN (DFOB) (2008) Price Schedules – June 2008. http://www.farmer.dfob.co.uk/Content/Price.asp

DAIRY UK (2008) Green and White: the environmental credentials of the dairy industry, Environmental Brochure, Dairy UK, London.

FOSS (2009) BactoScan FC. http://foss.co.uk/solutions/ProductsDirect/BactoScanFC.aspx

GALLMANN, P.U. and EBERHARD, P. (2004) New developments in heating technology for preservation and safety. In: *A Farm to Table Approach for Emerging and Developed Dairy Countries: Proceedings of IDF/FAO International Symposium on Dairy Safety and Hygiene,* Cape Town, 2–5 March, pp. 141–147.

GORDON, J.F. (1984) Importance and significance of raw milk quality to the liquid milk processor. *Journal of the Society of Dairy Technology* **37**(4), 141–145.

GRANT, I.R., HITCHINGS, E.I., MCCARTNEY, A., FERGUSON, F. and ROWE, M.T. (2002) Effect of commercial-scale high-temperature, short-time pasteurization on the viability of *Mycobacterium paratuberculosis* in naturally infected cow's milk. *Applied and Environmental Microbiology* **68**, 602–607.

GRANT, I.R., WILLIAMS, A.G., ROWE, M.T. and MUIR, D.D. (2005) Efficacy of various pasteurisation time–temperature conditions in combination with homogenisation on inactivation of *Mycobacterium avium* subsp *paratuberculosis* in milk. *Applied and Environmental Microbiology* **71**, 2853–2861.

HEGGUM, C. (2001) The reduction of MAP numbers in milk. Presented at Food Standards Agency Conference, London, 23–24 May.

HILLERTON, J.E. (2003) Control of MAP in milk. In: *Proceedings of the Conference on Quality Management at Farm Level. IDF World Dairy Summit and Centenary*, 7–12 September, Bruges, Belgium, pp. 263–267.

IDF (2004) *Guide to Good Dairy Farming Practice*. A joint publication of the International Dairy Federation and the Food and Agricultural Organisation of the United Nations, Rome, January.

IDF (2008) Guide *to Good Animal Welfare in Dairy Production*, International Dairy Federation, Brussels.

JOOSTE, P.J. and ANELICH, L.E.C.M. (2008) Safety and quality of dairy products. In: Britz, T.J. and Robinson, R.K. (eds) *Advanced Dairy Science and Technology*, pp. 153–178, Blackwell, Oxford.

O'MAHONEY, M., FANNING, S. and WHYTE, P. (2009) The safety of raw liquid milk. In: Tamime, A.Y. (ed.) *Milk Processing and Quality Management*, pp. 139–167, Blackwell, Oxford.

RENEAU, J.K., SEYKORA, A.J., HEINS, B.J., ENDRES, M.I., BEY, R.F. and FARNSWORTH, R.J. (2003) Relationship of cow hygiene scores and SCC. In: *Quality Counts Seminar, University of Minnesota, July 2003*, pp. 1–3.

TAFS (2007) Position paper on the safety of bovine milk and bovine milk products. *International Forum for Transmissible Animal Diseases and Food Safety*, http://www.tseandfoodsafety.org/position_papers/TAFS_POSITION_PAPER_ON_MILK.pdf

TE GIFFEL, M.C. (2003) Good hygienic practice in milk processing. In: Smit, G. (ed.) *Dairy*

Processing Improving Quality, pp. 68–80, Woodhead Publishing, Cambridge.

TSAKNIS, J. and LALAS, S. (2004) Chemical hazards an overview. In: *A Farm to Table Approach for Emerging and Developed Dairy Countries: Proceedings of the IDF/ FAO International Symposium on Dairy Safety and Hygiene*, Cape Town, 2–5 March, pp. 151–155.

USDA (2005) Food Safety and Inspection Service, United States Department of Agriculture, http://www.fsis.usda.gov/Fact_Sheets/Bovine_Spongiform_ Encephalopathy_Mad_Cow_Disease

4

Identifying pathogens in milk

B. Stessl and I. Hein, University of Veterinary Medicine Vienna, Austria

Abstract: This chapter is focused on reported outbreaks of infectious disease linked to consumption of contaminated milk and milk products. In many cases the pathogens are inactivated during the pasteurisation process, but re-contamination after pasteurisation or process failures bring about the presence of these pathogens in pasteurised milk and milk products. Furthermore, an extendend summary of current European, US and international regulations for micriobiological requirements in milk and milk products is provided. Sections 4.3 and 4.4 comprise an overview on conventional microbiological methods and additional new developed techniques for the detection and enumeration of the most relevant milkborne pathogens.

Key words: milkborne pathogen, regulation, conventional microbiological methods, new developed techniques.

4.1   Overview of milkborne pathogens

In developed countries it is estimated that up to one-third of the population are affected by microbiological foodborne diseases each year (Schlundt *et al.*, 2004). In England and Wales, 3% of all outbreaks reported to the national surveillance system of outbreaks of infectious intestinal disease from 1992 to 2000 were linked to consumption of contaminated milk and milk products (O'Brien *et al.*, 2006). Major contamination routes for raw milk are direct contact with contaminated sources in the dairy farm environment (e.g. soil, faeces, feed) and excretion from the udder of an infected animal. In many cases the pathogens are inactivated during the pasteurisation process, but

recontamination after pasteurisation or process failures bring about the presence of these pathogens in pasteurised milk and milk products (Oliver *et al*., 2005). Some of these pathogens such as *Listeria monocytogenes* are able to colonise dairy food processing plants, leading to long-term, intermittent contamination of the product (Gianfranceschi *et al*., 2006).

Segments of the population regard raw milk and raw milk products as more nutritious and health-promoting than pasteurised milk products, despite the fact that there is an increased risk of the presence of pathogens (Leedom, 2006), as many well-documented outbreaks involving the consumption of raw milk and raw milk products highlight. Concerning cheese made from raw milk, mainly fresh cheese and soft cheese with short duration of ripening, are of concern, since proper processing and ripening conditions minimise the risk of the presence of pathogens in long-ripened cheese (CDC, 2008a). The use of pasteurisation has reduced the number of milkborne diseases from 25% prior to 1938 to less than 1% nowadays in the USA (NEHA, 2008). Legislation concerning the distribution of raw milk and raw milk products differs between countries, as will be outlined later in this chapter. However, cow-sharing programmes involving the shared acquisition of a cow at a local farm (Denny *et al*., 2008) enable people to consume raw milk from that cow even in those countries where distribution of raw milk is illegal.

Although the number of sporadic cases is much higher, well-documented outbreaks of milkborne diseases provide important information about bacterial species and contamination routes involved. *Campylobacter jejuni*, *Salmonella* spp., Shiga toxin-producing *Escherichia coli* and *Listeria monocytogenes* are most frequently associated with milkborne outbreaks.

Campylobacteriosis presents as gastroenteritis with diarrhoea, abdominal cramps, headache, nausea and vomiting (Heuvelink *et al*., 2009). The neuropathic disorders Guillain–Barré and Miller–Fisher syndrome might develop after *C. jejuni* infections (Taboada *et al*., 2007). In most cases milk becomes contaminated from cow faeces, but a few cases of direct excretion of *C. jejuni* in the milk have been described (Hutchinson *et al*., 1985; Schildt *et al*., 2006). Both infection by drinking unpasteurised cow's and goat's milk and post-pasteurisation contamination have been described (Heuvelink *et al*., 2009; Lehner *et al*., 2000; Harris *et al*., 1987). In one case, contamination of raw milk was suspected to be due to incompletely sealed rubber liners fitted to a milking machine, allowing faecal material to contaminate the milk (Schildt *et al*., 2006). Post-pasteurisation contamination included birds pecking bottle tops and cross-contamination in the kitchen from a raw chicken (Riordan *et al*., 1993; Stuart *et al*., 1997; Jiménez *et al*., 2005).

Gastroenteritis caused by infection with *Salmonella* spp. is characterised by diarrhoea, abdominal cramps, fever, bloody stools and vomiting (Villar *et al*., 1999). Many cases involved consumption of raw milk – often Mexican-style – cheese made from cow's or goat's milk (Cody *et al*., 1999; Desenclos *et al*., 1996; De Valk *et al*., 2000; Villar *et al*., 1999). One case involved aged Mexican-style cheese suggesting processing failures during ageing of the cheese

(CDC, 2008a). Contaminated raw milk at a dairy-restaurant-petting zoo led to a multistate outbreak of salmonellosis with 62 confirmed cases in 2003 in the USA (CDC, 2003). Process failures such as defective pasteurisation and post-pasteurisation contamination due to high humidity and excessive condensation in the dairy plant as well as leakage of raw milk onto the floor were reported as other causes of salmonellosis (Anon., 1998; Olsen *et al.*, 2004). About 20 years ago a huge outbreak affecting more than 150,000 people was caused by a persistent strain in the plant, which repeatedly contaminated milk after pasteurisation (Ryan *et al.*, 1987). Contamination of powdered infant formula with *Salmonella* spp. and *Cronobacter sakazakii* is of special concern, with contamination occurring most often in the spray driers (vanAcker *et al.*, 2001; Cahill *et al.*, 2008; Soler *et al.*, 2008).

Similar to campylobacteriosis and salmonellosis, infections with Shiga toxin-producing *E. coli* are characterised by diarrhoea, bloody diarrhoea and abdominal cramps. In addition, haemolytic uremic syndrome might develop, requiring hospitalisation and in some cases ending fatally (Denny *et al.*, 2008; De Schrijver *et al.*, 2008). The main source of food contamination is bovine faeces. Most outbreaks involve *E. coli* O157:H7, whereas about half of the sporadic cases may be due to non-O157:H7 serotypes (Pradel *et al.*, 2008). Again, outbreaks involved on the one hand consumption of unpasteurised cow's and goat's milk and raw milk products and on the other hand pasteurisation failure and on-farm post-pasteurisation contamination (CDC, 2008b; Denny *et al.*, 2008; McIntyre *et al.*, 2008; Liptakova *et al.*, 2004; Goh *et al.*, 2002; De Schrijver *et al.*, 2008). One outbreak occurred due to consumption of unpasteurised Gouda cheese, despite it having met regulated microbiological and ageing requirements (Honish *et al.*, 2005).

Compared to campylobacteriosis and salmonellosis, the incidence of listeriosis is relatively low (0.1–11.3 per 1,000,000 inhabitants) but the average case-fatality rate of 20–30% is relatively high. Symptoms of the non-invasive form include febrile gastroenteritis, which occurs about 24 h after exposure and usually resolves spontaneously, and eczematous skin infection, which develops after direct contact with infected material, e.g. in veterinarians treating infected animals. The invasive form presents with focal infections most commonly involving the peritoneum, joints, endocardium or eyes, maternofoetal listeriosis or neonatal listeriosis, bloodstream infection and meningoencephalitis. In these cases the incubation period can exceed 30 days, thus hampering epidemiological investigations. Most of the cases are sporadic infections but there are some well-documented outbreaks reported in the literature linked to the consumption of contaminated milk products (Swaminathan and Gerner-Schmidt, 2007). Home-made or illicitly produced Mexican-style cheese was involved in some of them, as was post-pasteurisation contamination of pasteurised milk (CDC, 2001, 2007; MacDonald *et al.*, 2005). Similar to the other pathogens discussed so far, persistent strains in the environment also represent an important source of finished product contamination with special emphasis on persistence in dairy plants themselves (Gianfranceschi *et al.*, 2006; Kabuki *et al.*, 2004). For example, 3–17%

of red smear cheeses from different European countries were found to be contaminated with *L. monocytogenes* (Rudol and Scherer, 2001). Occasionally, subclinical udder infections occur, resulting in direct excretion of *L. monocytogenes* in the milk; for example, one outbreak with 120 cases arose due to consumption of fresh cheese made from unpasteurised raw goat's milk (Danielsson-Tham *et al.*, 2004; Schoder *et al.*, 2003).

Nowadays, infections with *Streptococcus equi* subsp. *zooepidemicus* are not very frequently encountered but cause severe illness (septicaemia, meningitis, endocarditis and glomerulonephritis) (Edwards *et al.*, 1988; Francis *et al.*, 1993). Outbreaks have been reported in different countries involving consumption of unpasteurised milk or cheese from goats and cows (Balter *et al.*, 2000; Bordes-Benítez *et al.*, 2006; Francis *et al.*, 1993; Kuusi *et al.*, 2006). Similarly, transmission of tick-borne encephalitis via consumption of raw milk and raw milk products occasionally occurs. Cases in different countries in Central and Eastern Europe were mainly associated with goat's milk (Donchenko *et al.*, 2005; Holzmann and Heinz, 2008; Matuszczyk *et al.*, 1997). Due to climate changes ticks are now present at higher altitudes. However, there is only a small window of time for excretion of virus in the milk, since the virus is quickly cleared from goats and human vaccination is an effective preventive measure (Holzmann and Heinz, 2008). Occasionally brucellosis has been contracted by consumption of unpasteurised raw milk or cheese from goats (Méndez Martínez *et al.*, 2003; Ramos *et al.*, 2008).

Concerning food poisonings linked to consumption of milk and milk products, *Staphylococcus aureus* and *Bacillus cereus* are the major culprits. The former produces heat-stable toxins whereas the latter produces heat-stable spores. Symptoms of food poisoning due to staphylococcal and *Bacillus cereus* toxins are similar and include vomiting, diarrhoea and abdominal cramps (McIntyre *et al.*, 2008; Jørgensen *et al.*, 2005). In both species not all strains produce toxins relevant for food poisoning.

Staphylococcal food poisoning is usually associated either with the presence of high numbers of toxin-producing *S. aureus* in milk before pasteurisation or with contamination with *S. aureus* after pasteurisation, e.g. by food workers or staff of restaurants or shops (Todd *et al.*, 2008). In the second case toxin production might start if food is kept for longer times at room temperature or mildly heated (Poli *et al.*, 2007). *S. aureus* may produce a wide array of different enterotoxins (SE), not all of them being characterised for their pathogenic potential up to now. SEH in mashed potatoes made with raw milk was the reason for an outbreak of staphylococcal food poisoning in Norway in 2005 and was also involved together with SEA in the massive Japanese outbreak due to contaminated reconstituted milk, which involved more than 10,000 people. Due to a power failure, the milk was kept for more than 9 h without refrigeration, enabling extensive toxin production. A few years ago a multistate outbreak (France, Switzerland, Germany, Austria and UK) was due to SE in French unpasteurised goat's cheese (Ikeda *et al.*, 2005; Jørgensen *et al.*, 2005; Soejima *et al.*, 2007; Espié and Vaillant, 2005).

Bacillus cereus is widely distributed in the environment, and high numbers of *B. cereus* in pasteurised milk were linked to teat contamination by soil (Christiansson *et al*., 1999). Spores survive the pasteurisation process, thus prevention of spore germination and vegetative proliferation by keeping milk and milk products at low temperature is important (Bartoszewicz *et al*., 2008). In addition, contamination during processing of pasteurised milk has been described (Svensson *et al*., 2000; Eneroth *et al*., 2001).

4.2   Regulatory aspects in Europe, the US and elsewhere

Milk from healthy cows contains relatively few bacteria (10^2–10^3 per ml), and the health risk from drinking raw milk would be minimal. During processing, milk is susceptible to contamination by many pathogenic microorganisms, which could result, depending on the virulence of the bacterial hazard, in a severe foodborne infection. Additionally, there is the potential that diseases of cows such as tuberculosis, brucellosis, typhoid and listeriosis can be transmitted (Spreer, 1998). Some countries such as the USA, Austria, Australia, New Zealand and Italy require absence of *L. monocytogenes*, e.g. in 25 g of foods (referred to as zero-tolerance) (UN Food and Agriculture Organization – FAO, 1999). This chapter provides an extended summary of European, US and international regulations for microbiological requirements in milk and milk products.

4.2.1   International aspects

The Codex Alimentarius is an international body that develops science-based food safety and commodity standards, guidelines and recommendations to promote consumer protection and to facilitate world trade (Wehr, 2004). Most important to the dairy industry are the Principles for the Establishment and Application of Microbiological Criteria for Foods, CAC/GL 21 (CAC, 1997), and the general standard Code of Hygienic Practice for Milk and Milk Products, CAC/RCP 57 (CAC, 2004).

4.2.2   European Commission Regulations for milk and milk products

According to the European Regulation on the General Principles and Requirements of Food Law, food business operators (FBOs) at all stages of production, processing and distribution within the businesses under their control shall ensure that foods satisfy the requirements of food law which are relevant to their activities and shall verify that such requirements are met (European Regulation 178/2002/EC – EC, 2002). International guidelines for microbiological criteria in respect of many foodstuffs have not yet been established. However, the Commission has followed the Codex Alimentarius guideline 'Principles for the establishment and application of microbiological criteria for foods, CAC/GL 21

Table 4.1 The food safety criteria and analytical reference methods of the European Regulation EC 2005/2073 for milk and milk products

Food category	Microorganisms	Sampling plan[a]	Limits[a] m M	Analytical reference method	Point of application
Cheeses, butter and cream from raw milk	*Salmonella*	$n = 5$, $c = 0$	Absence in 25 g	ISO 6579	End-product
Milk powder and whey powder	*Salmonella*	$n = 5$, $c = 0$	Absence in 25 g	ISO 6579	End-product
Ice cream	*Salmonella*	$n = 5$, $c = 0$	Absence in 25 g	ISO 6579	End-product
Cheese, milk powder and whey powder	Staphylococcal enterotoxins	$n = 5$, $c = 0$	Not detected in 25 g	Screening method of CRL[b]	End-product
Ready-to-eat foods able to support the growth of *L. monocytogenes*	*Listeria monocytogenes*	$n = 5$, $c = 0$	100 cfu/g	ISO 11290-2	Before product is placed on the market
Ready-to-eat foods unable to support the growth of *L. monocytogenes*	*Listeria monocytogenes*	$n = 5$, $c = 0$	Absence in 25 g	ISO 11290-1	End-product

[a] n = number of units comprising the sample, c = number of sample units giving values between m and M, where m = the acceptable microbiological level in a sample unit, M = the level which, when exceeded in one or more samples, would cause the lot to be rejected.
[b] CRL = Community Reference Laboratory.

– 1997', resulting in the new regulation EC 2073/2005. The latter contains microbiological criteria for specific food–microorganism combinations and the rules to be complied with by FBOs. A microbiological criterion defines the limit above which a food is considered to be contaminated at an unacceptable level with a microorganism, its toxin or metabolite and is therefore considered to be unsafe for consumption (European Regulation 2073/2005/EC – EC, 2005). There are two types of microbiological criteria. First, criteria affecting process hygiene are applicable to foodstuffs at various stages throughout their production processes and indicate whether or not the production process is operating in a hygienic manner. Additionally, food safety criteria are applicable to food placed on the market and throughout its shelf-life. They define the acceptability of a food in terms of its microbiological safety (Table 4.1).

4.2.3   US regulations on dairy products

The Food and Drug Administration (FDA) and the Department of Agriculture (USDA) are the two major US authorities that provide food safety standards for dairy products. The FDA recommends the application of the current Pasteurized Milk Ordinance (PMO) for Grade A Milk to unify national milk hygiene standards. The PMO is used as the sanitary regulation for milk and milk products served on interstate carriers, and is recognised by the Public Health Agencies, the milk industry and many others as the national standard for milk sanitation (US FDA, 2007). Furthermore, a Compliance Policy Guide provided by the FDA defines guidance for initiating legal action in cases involving products found to be improperly pasteurised, contaminated with pathogenic microorganisms, or prepared and packed under unsanitary conditions (US FDA, 2005; Table 4.2). In addition to FDA oversight of dairy product safety, many US dairy plants participate in a voluntary grading and inspection programme offered by the USDA through its Agricultural Marketing Service (AMS) (Anon., 2003).

Table 4.2   US FDA regulation on regulatory action levels for dairy products

Microorganism	Regulatory action level
Dairy products	
Salmonella, Campylobacter jejuni, Campylobacter coli, Yersinia enterocolitica, Listeria monocytogenes	≥ 1 positive unit
Staphylococcal enterotoxins	Positive
Cheese and cheese products	
Escherichia coli O 157:H7	$>10^3/g$
Escherichia coli	$>10^4/g$

Source: US Food and Drug Administration, Compliance policy guides, Chapter 5: Pathogens in dairy products.

4.2.4   Food standards in other countries

A strict regulation for microbiological limits in dairy products still exists in countries such as Australia and New Zealand (FSANZ, 2001). Raw milk samples for processing and cheese and cheese products, for example, should test negative for pathogens such as *Listeria monocytogenes*, *Salmonella* spp. and *Campylobacter* spp. In contrast to the US and Australian zero-tolerance policy, Canadian standards stipulate a maximum of 100 cfu/g for *L. monocytogenes* in foods that do not support the growth of the organism. Entirely, milk powder has to be tested negative for *Salmonella* spp. according to the Standards for Microbiological Safety of Food (Health Canada, 2008).

4.3   Current techniques for the detection of milkborne pathogens and their limitations

Traditional microbiological methods for detection and quantification of pathogenic bacteria in milk samples rely on growth on selective plating media, followed by isolation, biochemical and serological identification. Most European accredited food laboratories investigate milk and milk products for pathogenic bacterial contaminants according to ISO standard methods. In general, milk samples, initial suspensions and decimal dilutions should be prepared according to the guidance document for microbiological examination of milk and milk products (ISO 8261:2001 – ISO, 2001). The direct enumeration method of raw milk samples is appropriate for the determination of higher pathogen contamination levels (>100 cfu/ml). For this purpose, the surface of a selective agar is inoculated with 100 μl of the initial suspension and further decimal dilutions in duplicate. In the case of lower contamination levels (<100 cfu/ml), a selective enrichment step before plating onto agar is recommended. The most commonly used US standard methods for the examination of non-processed raw milk samples are those adopted by the American Public Health Association (APHA) and AOAC International. Furthermore, processed milk and milk products should be investigated according to the Food and Drug Administration's *Bacteriological Analytical Manual* (BAM) (US FDA, 2001).

An overview of conventional microbiological standard methods for the detection and enumeration of pathogenic bacteria in milk and food samples is presented in Table 4.3. Isolation and detection methods for pathogenic bacteria in food samples are laborious and time consuming, requiring 4–7 days to complete. Because of this, new plating media based on chromogenic substrates, which enhance the detection and differentiation of pathogenic bacteria and reduce the time and cost of analysis, have been introduced (Manafi, 2000; Perry and Freydière, 2007).

Conventional identification of typical colonies of milkborne pathogenic bacteria involves Gram-staining, catalase and oxidase test, motility, haemolysis and carbohydrate use. Therefore, it is recommended that at least five colonies be

identified by streaking the selected colonies onto the surface of nutrient agar plates. For further serological and biochemical confirmation, pure cultures have to be used. Additionally, rapid identification methods are used as screening techniques, with negative results accepted as is, but positive results requiring confirmation by the appropriate official method, which, in many instances, is cultural (Feng, 2001).

4.3.1 Conventional microbiological methods for the detection and enumeration of low numbers of milkborne pathogens

The minimum infective dose of *L. monocytogenes*, *Yersinia enterocolitica*, *Cronobacter sakazakii* and *Escherichia coli* O157 has not yet been determined. A *Salmonella* spp. infection could be caused by 15–20 cells, depending on the age and health of the host and strain differences among the members of the genus (US FDA, 2008). The recovery of low numbers of *L. monocytogenes* from food and environmental samples requires the use of enrichment cultures followed by selective plating (Beumer and Curtis, 2003). Successful isolation depends on the ability of the method to promote the growth of small numbers of potentially injured cells (e.g. $<10^2$ *Listeria*/cfu/ml), while at the same time minimising the growth of non-*Listeria* background organisms (Ryser and Donnelly, 2001). Additionally, the ability to isolate injured *Listeria* from food products may also be an important factor (Dykes and Withers, 1999).

4.4 New techniques for the detection of milkborne pathogens

A variety of different methods have been developed as alternatives to traditional microbiological detection and quantification of milkborne pathogens. A major reason for these developments was to decrease the time of the analysis. In addition, many of these methods enable high sample numbers to be processed in parallel. Different guidelines exist in different countries for validation of these methods, but all of them include testing the method in comparison to standard methods and determination of the rate of false negatives and false positives, the detection limit and, for quantitative methods, also the quantification limit (ISO, 2003; Feldsine *et al.*, 2002; Qvist, 2007).

4.4.1 Established methods

A wide range of non-commercial and commercial nucleic acid (DNA or RNA) or antibody–antigen reaction-based assays and combinations of both are available as fast alternative methods for the detection of pathogens in milk, a few of them being cited below. These methods are used for species identification of suspect colonies on agar plates, for detection of the pathogen in enrichment broth or for direct detection in milk and milk products. The last approach is more demanding, since it requires sophisticated methods for isolating the target from

Table 4.3 Conventional microbiological standard methods for the detection and enumeration of pathogen bacteria in milk and food samples

Pathogen	Organisation/Standard[a]	Pre-enrichment	Enrichment media	Plating media
Listeria monocytogenes	ISO 11290	Half Fraser	Fraser	ALOA (agar *Listeria* according to Ottaviani *et al.*, 1997) e.g. PALCAM agar
	NMKL 136	Half Fraser	Fraser	e.g. LMBA (*Listeria monocytogenes* blood agar medium)
	IDF 143 a	LEB (*Listeria* enrichment broth)	–	PALCAM agar Oxford agar
	FDA Chapter 10	BLEB (buffered *Listeria* enrichment broth)	BLEB (buffered *Listeria* enrichment broth + selective agents)	e.g. PALCAM agar e.g. MOX (Modified Oxford Medium) ALOA (agar *Listeria* according to Ottaviani *et al.*, 1997)
Staphylococcus aureus	ISO 6888 IDF 145 a NMKL 66	–	GBCTw (Giolitti–Cantoni broth + 1% Tween 80)	BP (Baird Parker agar) RPF (rabbit plasma fibrinogen agar)
	FDA Chapter 12	–	TSB (tryptic soy broth + 10% NaCl, 1% sodium pyruvate)	BP (Baird Parker agar)
Bacillus cereus	ISO 7932 IDF 181 NMKL No. 67 FDA Chapter 14	–	TSPB (tryptic soy broth + polymyxin)	MYP (Mannitol Egg Yolk Polymyxin agar) PEMBA (Polymyxin Pyruvate Egg Yolk Mannitol Bromthymol blue agar)
Salmonella spp.	ISO 6578 IDF 93 NMKL 71	BPW (buffered peptone water)	RVS (Rappaport–Vassiliardis Soya peptone) SC (selenite cystine medium)	BPLS (brilliant green phenol red lactose sucrose agar) e.g. XLD (xylose lysine desoxycholate agar)

	FDA Chapter 5	LB (lactose broth)	RV (Rappaport–Vassiliardis medium) TT (tetrathionate broth)	HE (Hektoen enteric agar) XLD (xylose lysine desoxycholate agar) BS (bismuth sulphite agar)
Campylobacter spp.	ISO 10272	Bolton broth	Bolton broth	mCCDA (Campy blood-free agar)
	FDA Chapter 7 NMKL No. 119	Bolton broth	Bolton broth	mCCDA (Campy blood-free agar) AHB (Abeyta–Hunt Bark agar)
Yersinia enterocolitica	ISO 10273 NMKL No. 117	–	PSB (peptone sorbitol bile broth) ITC (Irgasan Ticarcillin chlorate broth)	CIN (Cefsulodin–irgasan–novobiocin agar) SSDC (Salmonella-Shigelladeoxycholate calcium chloride agar) MacConkey agar
	FDA Chapter 8	–	PSB (peptone sorbitol bile broth)	CIN (Cefsulodin-irgasan-novobiocin agar)
Escherichia coli O157	ISO 16654 NMKL 164	–	mTSBn (tryptic soy broth + novobiocin) IMS (immuno-magnetic separation)	SMAC (Sorbitol MacConkey + Cefixime potassium)
	FDA Chapter 4a	BHI (brain heart infusion broth)	TP (tryptone phosphate broth)	L-EMB (Levine's eosin-methylene blue agar) MacConkey agar
Cronobacter sakazakii	ISO 22964 IDF RM 210	BPW (buffered peptone water)	mLST/vancomycin medium (modified lauryl sulphate tryptose broth + vancomycin)	ESIA (*Enterobacter sakazakii* isolation agar)

a FDA Food and Drug Administration; IDF International Dairy Federation; ISO International Standards Organization; NMKL Nordic Committee on Food Analysis.

the food matrix that is free from substances inhibiting the detection method (Stevens and Jaykus, 2004).

Polymerase chain reaction (PCR) is widely used and enables sensitive detection of pathogen-specific gene or RNA fragments. Real-time PCR generates a fluorescence signal if the gene is present in the sample, which is related to the number of target genes, thus enabling quantification also (Heid *et al*., 1996; McKillip and Drake, 2004; Whitman and Dunbar, 2008). Nowadays almost all commercial and many non-commercial PCR detection systems apply real-time PCR. In combination with an enrichment step, a detection limit of 5 cfu *Salmonella* and 1 cfu *C. jejuni* per 25 ml of milk was reported for this method (Hein *et al*., 2006; Oliveira *et al*., 2005). Real-time PCR was also used for detection and quantification of pathogens in milk and milk products, without performing an enrichment step prior to analysis. For direct detection of *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* in milk, a detection limit of 10^3 cfu/ ml, 10^2 cfu/0.5 ml and 7×10^1 cfu/ml respectively was reported, whereas in cheese the detection limit ranged from 2.0×10^1 to 3.5×10^2 per 2 g, depending on the cheese matrix (Hein *et al*., 2001, 2005; Yang *et al*., 2007; Singh *et al*., 2009).

A major obstacle of DNA-based PCR is the inability to discriminate between DNA from viable and dead cells or extracellular DNA (Reichert-Schwillinsky *et al*., 2009). In most cases only viable bacteria cause diseases. RNA is less stable than DNA so there is a better correlation between the presence of RNA and the presence of viable cells. However, 16S rRNA is too stable and with mRNA the target region has to be carefully selected for good correlation (Klein and Juneja, 1997; Norton and Batt, 1999). A disadvantage of the approach is the inability to quantify target bacteria due to differences in gene expression (Milner *et al*., 2001). Alternative RNA targets such as tmRNA might be more suitable (O'Grady *et al*., 2008).

A recent development is the application of monoazide-linked dyes such as ethidium bromide monoazide and propidium iodide monoazide for real-time PCR-based differentiation of viable and dead bacterial cells. These dyes selectively enter cells with damaged cell membranes and can be covalently linked to DNA via exposure to visible light, thus blocking PCR amplification. Selection of the dyes depends on the bacterial species (Flekna *et al*., 2007; Nogva *et al*., 2003). Activity-labile compounds were proposed as an alternative to that approach and could indicate cells with an active metabolism. These compounds should be membrane permeable and include a DNA-intercalating moiety, a crosslinking moiety and an intermediate linker, which could be cleaved by the enzymatic activity of the cell (Nocker and Camper, 2009).

Microarrays enable the parallel detection of large numbers of different pathogens via hybridisation of PCR-amplified DNA or RNA targets (Kim *et al*., 2008; Rasooly and Herold, 2008). This method was used for direct detection of *Yersinia enterocolitica* virulence genes in pasteurised whole milk with a detection limit of 1000 cfu per hybridisation and for identification and differentiation of *Enterococcus* spp. in artificially contaminated milk (Lehner *et al*.,

2005; Myers *et al*., 2006). Hybridisation in combination with magnetic capture and PCR was applied for the detection of as few as 10 cfu of *L. monocytogenes* per ml of milk. A 21-mer oligonucleotide specific for the *hlyA* gene was attached to magnetic nanoparticles and used for selective enrichment of *L. mono-cytogenes* DNA after DNA isolation, which was followed by PCR amplification (Amagliani *et al*., 2006).

Whole bacteria or toxins can be detected or recovered from milk for further analysis using antibodies, cell wall-binding domains (CBD) of phages or aptamers (single-stranded DNA or RNA ligands) and target-specific proteins (phage display technique), which are selected from large libraries of oligo-nucleotides or proteins with random sequences (Kretzer *et al*., 2007; Stratmann *et al*., 2006; Tombelli *et al*., 2007). Enzyme-linked immunosorbent assays (ELISA) are commonly used for the detection of staphylococcal enterotoxins (Bennett, 2005). The combination of immunoassays with PCR detection via nucleic acid-labelled secondary antibodies (immune-PCR) has the potential to enhance the detection limit of these methods (Adler *et al*., 2008). Paramagnetic CBD beads were used for separation of *L. monocytogenes* from 24-h enrichment broth and combined with selective plating for detection of 0.1 to 1 cfu/g cheese or ml milk (Kretzer *et al*., 2007). The performance of ssDNA aptamers was promising regarding viable/dead differentiation of bacteria using *Lactobacillus acidophilus* as a model (Hamula *et al*., 2008).

Another intriguing technique applies microspheres, which incorporate specific dyes. DNA or antibodies can be coupled to the surface and the attached target is marked via dye-labelled secondary antibodies or DNA fragments. The microspheres can be detected according to their colour and the intensity of the reaction on the surface measured by the additional dye label (Dunbar *et al*., 2003).

4.4.2   Future trends

Biosensors use a combination of a biological receptor (antibody, enzyme, nucleic acid, etc.) and a physical or physicochemical transducer to detect the analyte, which might be bacteria, toxins, DNA or RNA in the case of food hygiene. New technologies for biological receptors include aptamers (single-stranded DNA or RNA ligands) and target-specific proteins (phage display technique), which are selected from large libraries of oligonucleotides or proteins with random sequences. A broad variety of different designs have been developed in recent years, a few of them being presented below. Although these designs are promising, a major goal for future developments is to enhance the detection limit (Rasooly and Herold, 2006; Palchetti and Mascini, 2008).

Surface plasmon resonance biosensors (SPR) are some of the most commonly used biosensors (Rasooly and Herold, 2006). In SPR, energy carried by photons of light is transferred to electrons in metal. Thus all light is reflected except the resonant wavelength. Changes in mass at the surface are conferred by the presence of the target and cause a change in the resonant wavelength (Leonard *et*

al., 2004). SPR-based methods were used for the detection of *Salmonella* spp. and staphylococcal enterotoxins directly in milk, with a detection limit of 10^5 cfu/ml and 0.5 ng/ml, respectively (Homola *et al*., 2002; Mazdumar *et al*., 2007).

Lin *et al*. (2008) used screen-printed carbon electrodes with gold nano-particles and monoclonal anti-*E. coli* O157:H7 antibodies attached to the surface to detect this bacterium in milk. Ferrocenedicarboxylic acid was used as the electrochemically active analyte to mediate the H_2O_2-peroxidase reaction of the horseradish peroxidase-conjugated sandwich antibody to the working electrode. The detection limit of this method was 10^3 cfu/ml milk.

A magnetoelastic biosensor was used for the detection of *Salmonella* Typhimurium and *Bacillus anthracis* spores (Huang *et al*., 2008). A time-varying magnetic field was applied to a sensor platform, inducing vibration of the platform with the greatest amplitude at a characteristic frequency of the magnetic field; the vibration is converted into a change of current. Phages were used for immobilisation of the target cells or spores. The capture of targets changed the characteristic frequency of the vibration.

Gold electrodes with a self-assembled monolayer and attached antibodies were used for qualitative discrimination of *E. coli* and *Salmonella* Typhimurium in milk (Mantzila *et al*., 2008). The targets induce a change in impedance on the surface of the electrode upon binding to the antibodies.

Cell-based biosensors record the physiological response of a living cell or cellular component to external stimuli (Banerjee *et al*., 2008). B lymphocytes immobilised in a collagen matrix in microtitre plates were used to detect *L. monocytogenes* cells and toxin preparations from *L. monocytogenes* and *B. cereus*. Toxicity was measured by the release of alkaline phosphatase from the infected cells. Rider *et al*. (2003) used pathogen-specific B cell lines with stable cytosolic expression of the photoprotein aequorin, which emit light upon binding to the specific pathogen. This technology is called CANARY (cellular analysis and notification of antigen risks and yields) and was used for the detection of *E. coli* O157:H7.

Some technologies include bacteriophages, which are viruses that infect bacteria. *Escherichia coli* was detected by using a modified phage for infection, which triggered the generation of a signalling molecule by the infected cell (acyl-homoserine lactone), inducing bioluminescence in a bioreporter bacterium (Ripp *et al*., 2006). Edgar *et al*. (2006) used a phage for the detection of bacteria, which displays a special protein when produced by the bacteria upon infection. The host cell's biotin-ligase protein attaches biotin to this protein and the biotin-labelled phages are detected by streptavidin quantum-dot nanocomplexes using fluorescence measurement (flow cytometry).

Biosensors might also be used for specific detection of DNA or RNA. An E-DNA sensor was described, employing a molecular beacon modified with a redox label and attached to an electrode via alkanethiol self-assembled mono-layer chemistry. Upon hybridisation to DNA or RNA the beacon unfolds and the redox tag is forced away from the electrode, producing a reduction in redox current (Lubin *et al*., 2006). A so-called genosensor array was used to detect *L.*

monocytogenes, *Salmonella* spp., *S. aureus* and *E. coli* O157:H7 in a sandwich hybridisation format (capture probe attached to gold electrode and a signalling probe) with voltammetric detection via the label of the signalling probe (Farabullini *et al.*, 2007). Chen *et al.* (2008) used a probe attached to a piezo-electric biosensor surface to detect the foodborne pathogen *E. coli* O157:H7. A specific region in the *eaeA* gene was amplified by PCR and hybridised to the immobilised probe. The resulting mass change was amplified by a second probe conjugated to gold nanoparticles used as mass enhancer (sandwich hybridisation format) and resulted in a frequency change of the piezoelectric biosensor.

More consumer-oriented new developments include pathogen indicators based on the concept of active and intelligent packaging. The packaging material harbours a pathogen detection zone (e.g. antibodies) changing colour upon contact with the pathogen, which can easily be detected by the consumer. This system was proposed by a UK-based dairy company as a freshness indicator, turning from white to red as the level of lactic acid increases (De Jong *et al.*, 2005; Oltes and Yalcin, 2008).

4.5   Sources of further information and advice

4.5.1   For Section 4.1

FRATAMICO P M, BHUNIA A K and SMITH J L (2005), *Foodborne Pathogens: Microbiology and Molecular Biology*, Norfolk, UK, Caister Academic Press.

MILLIOTIS M D and BIER J W (2003), *International Handbook of Foodborne Pathogens*, Boca Raton, FL, CRC Press.

http://www.foodrisk.org
http://www.cdc.gov/FoodNet
http://www.foodnetbase.com
http://www.foodsafety.gov
http://www.cfsan.fda.gov
http://www.efsa.europa.eu
http://ec.europa.eu/food/index_en.htm
http://www.eufic.org
http://ecdc.europa.eu/en
http://www.foodstandards.gov.au
http://www.safefood.net.au
http://www.aifst.asn.au

4.5.2   For Section 4.2

WEHR M and FRANK J F (2004), *Standard Methods for the Examination of Dairy Products*, Washington, DC, American Public Health Association.

DOWNES F P and ITO K (2001), *Compendium of Methods for the Microbiological Examination of Foods*, Washington, DC, American Public Health Association.

SPREER E (1998)*, Milk and Dairy Product Technology*, New York, Marcel Dekker.

EC (2004), Commission Regulation 882/2004/EC of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal

health and animal welfare rules, *Official Journal of the European Communities*, L191, 1–52.

http://www.codex.alimentarius.org

http://ec.europa.eu/food/index_en.htm

http://www.fao.org

http://www.foodsafety.gov

http://www.foodstandards.gov.au

http://www.fsis.usda.gov

http://www.hc-sc.gc.ca

4.5.3   For Section 4.3

CORRY J E L, CURTIS G D W and BAIRD R M (2003), *Handbook of Culture Media for Food Microbiology*, Amsterdam, Elsevier.

http://www.aoac.org

http://www.fsan.fda.gov/bam

http://www.iso.org

http://www.fil-idf.org/Content/Default.asp

http://www.nmkl.org/

ISO 6579 (2002), Microbiology of food and animal feeding stuffs, Horizontal method for the detection of *Salmonella* spp.

ISO 8261 (2001), Milk and milk products – General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination.

ISO 6888-1 (1999), Microbiology of food and animal feeding stuffs, Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium.

ISO 6888-2 (1999), Microbiology of food and animal feeding stuffs, Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 2: Technique using rabbit plasma fibrinogen agar medium.

ISO 7932 (2004), Microbiology of food and animal feeding stuff, Horizontal method for the enumeration of presumptive *Bacillus cereus*, Colony-count technique at 30 degrees C.

ISO 10272-1 (2006), Microbiology of food and animal feeding stuff, Horizontal method for detection and enumeration of *Campylobacter* spp – Part 1: Detection method.

ISO/TS 10272-2 (2006), Microbiology of food and animal feeding stuffs, Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 2: Colony-count technique.

ISO 10273 (2003), Microbiology of food and animal feeding stuffs, Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica.*

ISO 11290-1 (1996), Microbiology of food and animal feeding stuffs, Horizontal method for the detection and enumeration of *Listeria monocytogenes*, Part 1: Detection method.

ISO 11290-1 (1996/Amd 1:2004), Modification of the isolation media and the haemolysis test, and inclusion of precision data.

ISO 11290-2 (1998), Microbiology of food and animal feeding stuffs, Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 2: Enumeration method.

ISO 16654 (2001), Microbiology of food and animal feeding stuffs, Horizontal method for the detection of *Escherichia coli* O157.

ISO 6785 (2001), Milk and milk products, Detection of *Salmonella* spp.

ISO/TS 22964 (2006), Milk and milk products, Detection of *Enterobacter sakazakii.*

IDF Standard 93B (1995), Milk and milk products, Detection of *Salmonella.*

IDF standard 143A (1995), Milk and milk products, Detection of Listeria monocytogenes.

IDF Standard 145A (1997), Milk and milk-based products – Enumeration of coagulase-positive staphylococci – Colony count technique at 37ºC.

IDF Standard 181 (1998), Dried milk products. Enumeration of *Bacillus cereus*, Most probable number technique.

FENG P and WEAGANT S D (2002), Diarrheagenic *Escherichia coli*, Chapter 4a in *Bacteriological Analytical Manual*.

ANDREWS W H and HAMMACK T S (2007), Salmonella, Chapter 5 in *Bacteriological Analytical Manual*.

HUNT J M, ABEYTA C and TRAN T (2001), *Campylobacter* spp., Chapter 7 in *Bacteriological Analytical Manual*.

WEAGANT S D and FENG P (2007), *Yersinia enterocolitica*, Chapter 8 in *Bacteriological Analytical Manual*.

HITCHINS A D (2003), Detection and enumeration of Listeria monocytogenes in foods, Chapter 10 in *Bacteriological Analytical Manual*.

BENNET R W and LANCETTE G A (2001), Staphylococcus aureus, Chapter 12 in *Bacteriological Analytical Manual*.

RHODEHAMEL E J and HARMON S M (2001), Bacillus cereus, Chapter 14 in *Bacteriological Analytical Manual*.

NMKL 66 (1999), Coagulase positive staphylococci, Enumeration in foods.

NMKL 67 (1997), *Bacillus cereus*, Determination in foods.

NMKL 71 (1999), Salmonella, Detection in foods.

NMKL 117 (1996), *Yersinia enterocolitica*, Detection in foods.

NMKL 119 (1990), *Campylobacter jejuni/coli*, Detection in foods.

NMKL 136 (2004), *Listeria monocytogenes*, Detection in foods and feeds.

NMKL 151(1995), *Shigella* bacteria, Detection in foods.

NMKL 156 (1997), Pathogenic *Vibrio* species, Detection and enumeration in foods.

NMKL 164 (1999), *Escherichia coli* O157, Detection in food and feeding stuffs

4.5.4   For Section 4.4

MAURER J (2006), *Food Microbiology and Food Safety Series: PCR Methods in Foods*, New York, Springer.

MACKAY I M (2007), *Real-time PCR in Microbiology: From Diagnosis to Characterization*, Norwich, UK, Caister Academic Press.

ADLEY C C (2006), *Food-borne Pathogens: Methods and Protocols*, Totowa, NJ, Humana Press.

http://www.rapidmethod.com

http://www.rapidmicrobiology.com

http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php

4.6   References

ADLER M, WACKER R and NIEMEYER C M (2008), 'Sensitivity by combination: immuno-PCR and related technologies', *Analyst*, 133, 702–718.

AMAGLIANI G, OMICCIOLI E, DEL CAMPO A, BRUCE I J, BRANDI G and MAGNANI M (2006), 'Development of a magnetic capture hybridization-PCR assay for *Listeria monocytogenes* direct detection in milk samples', *J Appl Microbiol*, 100, 375–383.

ANON. (1998), 'Defective pasteurisation linked to outbreak of *Salmonella* Typhimurium definitive phage type 104 infection in Lancashire', *Commun Dis Rep CDR Wkly*, 8, 335–338.

ANON. (2003), 'Scientific criteria to ensure safe food', National Research Council Committee, Washington, DC, National Academic Science. Available from http://www.nap.edu/catalog/10690.htm [accessed 4 July 2009].

BALTER S, BENIN A, PINTO S W L, TEIXEIRA L M, ALVIM G G, LUNA E, JACKSON D, LACLAIRE L, ELLIOTT J, FACKLAM R and SCHUCHAT A (2000), 'Epidemic nephritis in Nova Serrana, Brazil', *Lancet*, 355, 1776–1780.

BANERJEE P, LENZ D, ROBINSON J P, RICKUS J L and BHUNIA A K (2008), 'A novel and simple cell-based detection system with a collagen-encapsulated B-lymphocyte cell line as a biosensor for rapid detection of pathogens and toxins', *Lab Invest*, 88, 196–206.

BARTOSZEWICZ M, HANSEN B M and SWIECICKA I (2008), 'The members of *Bacillus cereus* group are commonly present contaminants of fresh and heat-treated milk', *Food Microbiol*, 25, 588–596.

BENNETT R W (2005), 'Staphylococcal enterotoxin and its rapid identification in foods by enzyme-linked immunosorbent assay-based methodology', *J Food Prot*, 68, 1264–1270.

BEUMER R R and CURTIS G D W (2003), 'Culture media and methods for the isolation of *Listeria monocytogenes*', Chapter 5, in Corry J E L *et al*., *Handbook of Culture Media for Food Microbiology*, Amsterdam, Elsevier, 79–90.

BORDES-BENÍTEZ A, SÁNCHEZ-OÑORO M, SUÁREZ-BORDÓN P, GARCÍA-ROJAS A J, SAÉZ-NIETO J A, GONZÁLEZ-GARCÍA A, ÁLAMO-ANTÚNEZ I, SÁNCHEZ-MAROTO A and BOLAÑOS-RIVERO M (2006), 'Outbreak of *Streptococcus equi* subsp. *zooepidemicus* infections on the island of Gran Canaria associated with consumption of inadequately pasteurized cheese', *Eur J Clin Microbiol Infect Dis*, 25, 242–246.

CAC (1997), Principles for the Establishment and Application of Microbiological Criteria for Foods, *Codex Alimentarius Commission*, CAC/GL 21, 1–4.

CAC (2004), Code of Hygienic Practice for Milk and Milk Products, *Codex Alimentarius Commission*, CAC/RCP 57, 1–40.

CAHILL S M, WACHSMUTH I K, COSTARRICA M and EMBAREK P K B (2008), 'Powdered infant formula as a source of *Salmonella* infection in infants', *Clin Infect Dis*, 46, 268–273.

CDC (2001), 'Outbreak of listeriosis associated with homemade Mexican-style cheese – North Carolina, October 2000–January 2001', *MMWR Morb Mortal Wkly Rep*, 50, 560–562.

CDC (2003), 'Multistate outbreak of *Salmonella* serotype Typhimurium infections associated with drinking unpasteurised milk – Illinois, Indiana, Ohio and Tennessee, 2002–2003', *MMWR Morb Mortal Wkly Rep*, 52, 613–615.

CDC (2007), 'Outbreak of *Listeria monocytogenes* infections associated with pasteurized milk from a local dairy – Massachusetts, 2007', *MMWR Morb Mortal Wkly Rep*, 57, 1097–1100.

CDC (2008a), 'Outbreak of multidrug-resistant *Salmonella enterica* serotype Newport infections associated with consumption of unpasteurized Mexican-style aged cheese – Illinois, March 2006–April 2007', *MMWR Morb Mortal Wkly Rep*, 57, 432–435.

CDC (2008b), '*Escherichia coli* O157:H7 infections in children associated with raw milk and raw colostrums from cows – California, 2006', *MMWR Morb Mortal Wkly Rep*, 57, 625–628.

CHEN S H, WU V C H, CHUANG Y C and LIN C S (2008), 'Using oligonucleotide-functionalized Au nanoparticles to rapidly detect foodborne pathogens on a piezoelectric biosensor', *J Microbiol Meth*, 73, 7–17.

CHRISTIANSSON A, BERTILSSON J and SVENSSON B (1999), '*Bacillus cereus* spores in raw milk: factors affecting the contamination of milk during the grazing period', *J Dairy Sci*, 82, 305–314.

CODY S H, ABBOTT S L, MARFIN A A, SCHULZ B, WAGNER P, ROBBINS K, MOHLE-BOETANI J C and VUGIA D J (1999), 'Two outbreaks of multidrug-resistant *Salmonella* serotype Typhimurium DT104 infections linked to raw-milk cheese in northern California', *JAMA*, 281, 1805–1810.

DANIELSSON-THAM M L, ERIKSSON E, HELMERSSON S, LEFFLER M, LÜDTKE L, STEEN M, SØRGJERD S and THAM M (2004), 'Causes behind a human cheese-borne outbreak of gastrointestinal listeriosis', *Foodborne Pathog Dis*, 1, 153–159.

DE JONG A R, BOUMANS H, SLAGHEK T, VAN VEEN J, RIJK R and VAN ZANDVOORT M (2005), 'Active and intelligent packaging of food: Is it the future?', *Food Addit Contam*, 22, 975–979.

DE SCHRIJVER K, BUVENS G, POSSÉ B, VAN DEN BRANDEN D, OOSTERLYNCK O, DE ZUTTER L, EILERS K, PIÉRARD D, DIERICK K, VAN DAMME-LOMBAERTS R, LAUWERS C and JACOBS R (2008), 'Outbreak of verocytotoxin-producing *E. coli* O145 and O26 infections associated with the consumption of ice cream produced at a farm, Belgium, 2007', *Euro Surveill*, 13, 8041.

DE VALK H, DELAROCQUE-ASTAGNEAU E, COLOMB G, PLE S, GODARD E, VAILLANT V, HAEGHEBAERT S, BOUVET P H, GRIMONT F, GRIMONT P and DESENCLOS J C (2000), 'A community-wide outbreak of *Salmonella enterica* serotype Typhimurium infection associated with eating a raw milk soft cheese in France', *Epidemiol Infect*, 142, 1–7.

DENNY J, BHAT M and ECKMANN K (2008), 'Outbreak of *Escherichia coli* O157:H7 associated with raw milk consumption in the pacific northwest', *Foodborne Pathog Dis*, 5, 321–328.

DESENCLOS J C, BOUVET P, BENZ-LEMOINE E, GRIMONT F, DESQUEYROUX H, REBIÈRE I and GRIMONT P A (1996), 'Large outbreak of *Salmonella enterica* serotype Paratyphi B infection caused by a goat's milk cheese, France, 1993: a case finding and epidemiological study', *BMJ*, 312, 91–94.

DONCHENKO I, KUTSAR K and VASILENKO V (2005), 'Tickborne encephalitis outbreak in Estonia linked to raw goat milk, May–June 2005', *Euro Surveill*, 10, 2730.

DUNBAR S A, VANDER ZEE C A, OLIVER K G, KAREM K L and JACOBSON J W (2003), 'Quantitative, multiplexed detection of bacterial pathogens: DNA and protein applications of the Luminex LabMAP^{TM} system', *J Microbiol Meth*, 53, 245–252.

DYKES G A and WITHERS K M (1999), 'Sub-lethal damage of *Listeria monocytogenes* after long-term chilled storage at 4ºC', *Lett Appl Microbiol*, 28, 45–48.

EC (2002), Commission Regulation 178/2002/EC of 28 January 2002 on general principles and requirements of food law, establishing the European Food Safety

Authority and laying down procedures in matters of food safety, *Official Journal of the European Communities*, L31, 1–24.

EC (2005), Commission Regulation 2073/2005/EC of 15 November 2005 on microbiological criteria for foodstuffs, *Official Journal of the European Communities*, L338, 1–26.

EDGAR R, MCKINSTRY M, HWANG J, OPPENHEIM A B, FEKETE R A, GIULIAN G, MERRIL C, NAGASHIMA K and ADHYA S (2006), 'High-sensitivity bacterial detection using biotin-tagged phage and quantum-dot nanocomplexes', *PNAS*, 103, 4841–4845.

EDWARDS A T, ROULSON M and IRONSIDE M J (1988), 'A milk-borne outbreak of serious infection due to *Streptococcus zooepidemicus* (Lancefield group C)', *Epidemiol Infect*, 101, 43–51.

ENEROTH Å, SVENSSON B, MOLIN G and CHRISTIANSSON A (2001), 'Contamination of pasteurized milk by *Bacillus cereus* in the filling machine', *J Dairy Res*, 68, 189–196.

ESPIÉ E and VAILLANT V (2005), 'International outbreak of *Salmonella* Stourbridge infection, April–July 2005: results of epidemiological, food and veterinary investigations in France', *Euro Surveill*, 32, 2772.

FAO (1999), 'Report of the FAO Expert Consultation on the Trade Impact of *Listeria* in Fish Products', Rome, Food and Agriculture Organization of the United Nations. Available from http://www.fao.org/docrep/003/x3018e/X3018E06.htm [accessed 2 July 2009].

FARABULLINI F, LUCARELLI F, PALCHETTI I, MARRAZZA G and MASCINI M (2007), 'Disposable electrochemical genosensor for the simultaneous analysis of different bacterial food contaminants', *Biosens Bioelectron*, 22, 1544–1549.

FELDSINE P, ABEYTA C and ANDREWS W H, AOAC INTERNATIONAL METHODS COMMITTEE (2002), 'AOAC International Methods Committee guidelines for validation of qualitative and quantitative food microbiological office methods of analysis', *J AOAC Int*, 85, 1187–1200.

FENG P (2001), 'Appendix 1, Rapid Methods for Detecting Foodborne Pathogens', *Food and Drug Administration's Bacteriological Analytical Manual*. Available from http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/Bacteriological AnalyticalManualBAM/default.htm [accessed 2 July 2009].

FLEKNA G, ŠTEFANIČ P, WAGNER M, SMULDERS F J M, SMOLE MOŽINA S and HEIN I (2007), 'Insufficient differentiation of live and dead *Campylobacter jejuni* and *Listeria monocytogenes* cells by ethidium monoazide (EMA) compromises EMA/real-time PCR', *Res Microbiol*, 158, 405–412.

FRANCIS A J, NIMMO G R, EFSTRATIOU A, GALANIS V and NUTTALL N (1993), 'Investigation of milk-borne *Streptococcus zooepidemicus* infection associated with glomerulonephritis in Australia', *J Infect*, 27, 317–323.

FSANZ (2001), User Guide to Standard 1.6.1 – Microbiological limits for food with additional guideline criteria', Canberra, Australia and Wellington, New Zealand, *Food Standards Australia New Zealand*, 1–22.

GIANFRANCESCHI M, D'OTTAVIO M C, GATTUSO A, POURSHABAN M, BERTOLETTI I, BIGNAZZI R, MANZONI P, MARCHETTI M and AURELI P (2006), 'Listeriosis associated with Gorgonzola (Italian blue-veined cheese)', *Foodborne Pathog Dis*, 3, 190–195.

GOH S, NEWMAN C, KNOWLESS M, BOLTON F J, HOLLYOAK V, RICHARDS S, DALEY P, COUNTER D, SMITH H R and KEPPIE N (2002), '*E. coli* O157 phage type 21/28 outbreak in North Cumbria associated with pasteurized milk', *Epidemiol Infect*, 129, 451–457.

HAMULA C L A, ZHANG H, GUAN L L, LI X F and LE X C (2008), 'Selection of aptamers against

live bacterial cells', *Anal Chem*, 80, 7812–7819.

HARRIS N V, KIMBALL T J, BENNETT P, JOHNSON Y, WAKELY D and NOLAN C M (1987), '*Campylobacter jejuni* enteritis associated with raw goat's milk', *Am J Epidemiol*, 126, 179–186.

HEALTH CANADA (2008), 'Standards and Guidelines for Microbiological Safety of Food, an Interpretive Summary', Ottawa, Health Canada and Food Directorate, Health Protection Branch, 1–16.

HEID C A, STEVENS J, LIVAK K J and WILLIAMS P M (1996), 'Real-time quantitative PCR', *Genome Res*, 6, 986–994.

HEIN I, LEHNER A, RIECK P, KLEIN K, BRANDL E and WAGNER M (2001), 'Comparison of different approaches to quantify *Staphylococcus aureus* cells by real-time quantitative PCR and application of this technique for examination of cheese', *Appl Environ Microbiol*, 67, 3122–3126.

HEIN I, JØRGENSEN H J, LONCAREVIC S and WAGNER M (2005), 'Quantification of *Staphylococcus aureus* in unpasteurised bovine and caprine milk by real-time PCR', *Res Microbiol*, 156, 554–563.

HEIN I, FLEKNA G, KRASSNIG M and WAGNER M (2006), 'Real-time PCR for the detection of *Salmonella* spp. in food: an alternative approach to a conventional PCR system suggested by the FOOD-PCR project', *J Microbiol Meth*, 66, 538–547.

HEUVELINK A E, VAN HEERWAARDEN C, ZWARTKRUIS-NAHUIS A, TILBURG J J, BOS M H, HEILMANN F G, HOFHUIS A, HOEKSTRA T and DE BOER E (2009), 'Two outbreaks of campylobacteriosis associated with the consumption of raw cows' milk', *Int J Food Microbiol*, 134, 70–74.

HOLZMANN H and HEINZ F X (2008), 'FSME-Ausbruch durch Konsum von Frischkäse aus nichtpasteurisierter Ziegenmilch', *ImpfDialog*, 3, 105–106.

HOMOLA J, DOSTÁLEK J, CHEN S, RASOOLY A, JIANG S and YEE S S (2002), 'Spectral surface plasmon resonance biosensor for detection of staphylococcal enterotoxin B in milk', *Int J Food Microbiol*, 75, 61–69.

HONISH L, PREDY G, HISLOP N, CHUI L, KOWALEWSKA-GROCHOWSKA K, TROTTIER L, KREPLIN C and ZAZULAK I (2005), 'An outbreak of *E. coli* O157:H7 hemorrhagic colitis associated with unpasteurized gouda cheese', *Can J Public Health*, 96, 182–184.

HUANG S, YANG H, LAKSHMANAN R S, JOHNSON M L, WAN J, CHEN I H, WIKLE III H C, PETRENKO V A, BARBAREE J M and CHIN B A (2008), 'Sequential detection of *Salmonella typhimurium* and *Bacillus anthracis* spores using magnetoelastic biosensors', *Biosens Bioelectron*, doi:10.1016/j.bios.2008.09.006.

HUTCHINSON D N, BOLTON F J, JELLEY W C, MATHEWS W G, TELFORD D R, COUNTER D E, JESSOP E G and HORSLEY S D (1985), 'Campylobacter enteritis associated with consumption of raw goat's milk', *Lancet*, 1, 1037–1038.

IKEDA T, TAMATE N, YAMAGUCHI K and MAKINO S (2005), 'Mass outbreak of food poisoning disease caused by small amounts of staphylococcal enterotoxins A and H', *Appl Environ Microbiol*, 71, 2793–2795.

ISO (2001), 'Milk and milk products – General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination, ISO 8261:2001', Geneva, International Organization for Standardization.

ISO (2003), 'Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods, ISO 16140:2003', Geneva, International Organization for Standardization.

JIMÉNEZ M, SOLER P, VENANZI J D, CANTÉ P, VARELA C and MARTÍNEZ NAVARRO F (2005), 'An

outbreak of *Campylobacter jejuni* enteritis in a school of Madrid, Spain', *Euro Surveill*, 10, 118–121.

JØRGENSEN H J, MATHISEN T, LØVSETH A, OMOE K, QVALE K S and LONCAREVIC S (2005), 'An outbreak of staphylococcal food poisoning caused by enterotoxin H in mashed potato made with raw milk', *FEMS Microbiol Lett*, 252, 267–272.

KABUKI D Y, KUAYE A Y, WIEDMANN M and BOOR K J (2004), 'Molecular subtyping and tracking of *Listeria monocytogenes* in Latin-style fresh-cheese processing plants', *J Dairy Sci*, 87, 2803–2812.

KIM H J, PARK S H, LEE T H, NAHM B H, KOM Y R and KIM H Y (2008), 'Microarray detection of food-borne pathogens using specific probes prepared by comparative genomics', *Biosens Bioelectron*, 24, 238–246.

KLEIN P G and JUNEJA V K (1997), 'Sensitive detection of viable *Listeria monocytogenes* by reverse transcription-PCR', *Appl Environ Microbiol*, 63, 4441–4448.

KRETZER J W, LEHMANN R, SCHMELCHER M, BANZ M, KIM K P, KORN C and LOESSNER M J (2007), 'Use of high-affinity cell wall-binding domains of bacteriophage endolysins for immobilization and separation of bacterial cells', *Appl Environ Microbiol*, 73, 1992–2000.

KUUSI M, LAHTI E, VIROLAINEN A, HATAKKA M, VUENTO R, RANTALA L, VUOPIO-VARKILA J, SEUNA E, KARPPELIN M, HAKKINEN M, TAKKINEN J, GINDONIS V, SIPONEN K and HUOTARI K (2006), 'An outbreak of *Streptococcus equi* subspecies *zooepidemicus* associated with consumption of fresh goat cheese', *BMC Infect Dis*, 6, 36.

LEEDOM J M (2006), 'Milk of nonhuman origin and infectious diseases in humans', *Clin Infect Dis*, 43, 610–615.

LEHNER A, SCHNECK C, FEIERL G, PLESS P, DEUTZ A, BRANDL E and WAGNER M (2000), 'Epidemiologic application of pulsed-field gel electrophoresis to an outbreak of *Campylobacter jejuni* in an Austrian youth centre', *Epidemiol Infect*, 125, 13–16.

LEHNER A, LOY A, BEHR T, GAENGE H, LUDWIG W, WAGNER M and SCHLEIFER K H (2005), 'Oligonucleotide microarray for identification of *Enterococcus* species', *FEMS Microbiol Lett*, 246, 133–142.

LEONARD P, HEARTY S, QUINN J and O'KENNEDY R (2004), 'A generic approach for the detection of whole *Listeria monocytogenes* cells in contaminated samples using surface plasmon resonance', *Biosens Bioelectron*, 19, 1331–1335.

LIN Y H, CHEN S H, CHUANG Y C, LU Y C, SHEN T Y, CHANG C A and LIN C S (2008), 'Disposable amperometric immunosensing strips fabricated by Au nanoparticles – modified screen-printed carbon electrodes for the detection of foodborne pathogen *Escherichia coli* O157:H7', *Biosens Bioelectron*, 23, 1832–1837.

LIPTAKOVA A, SIEGFRIED L, ROSOCHA J, PODRACKA L, BOGYIOVA E and KOTULOVA D (2004) 'A family outbreak of haemolytic uraemic syndrome and haemorrhagic colitis caused by verocytotoxigenic *Escherichia coli* O157 from unpasteurised cow's milk in Slovakia', *Clin Microbiol Infect*, 10, 574–592.

LUBIN A A, LAI R Y, BAKER B R, HEEGER A J and PLAXCO K W (2006), 'Sequence-specific, electronic detection of oligonucleotides in blood, soil, and foodstuffs with the reagentless, reusable E-DNA sensor', *Anal Chem*, 78, 5671–5677.

MACDONALD P D M, WHITWAM R E, BOGGS J D, MACCORMACK J N, ANDERSON K L, REARDON J W, SAAH J R., GRAVES L M, HUNTER S B and SOBEL J (2005), 'Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese', *Clin Infect Dis*, 40, 677–682.

MANAFI M (2000), 'New developments in chromogenic and fluorogenic culture media', *Int J Food Microbiol*, 60, 205–218.

MANTZILA A G, MAIPA V and PRODROMIDIS M I (2008), 'Development of a faradic impedimetric immunosensor for the detection of *Salmonella typhimurium* in milk', *Anal Chem*, 80, 1169–1175.

MATUSZCZYK I, TARNOWSKA H, ZABICKA J and GUT W (1997), 'The outbreak of an epidemic of tick-borne encephalitis in Kielec province induced by milk ingestion', *Przegl Epidemiol*, 51, 381–388.

MAZDUMAR S D, HARTMANN M, KÄMPFER P and KEUGSEN M (2007), 'Rapid method for detection of *Salmonella* in milk by surface plasmon resonance (SPR)', *Biosens Bioelectron*, 22, 2040–2046.

MCINTYRE L, BERNARD K, BENIAC D, ISAAC-RENTON J L and NASEBY D C (2008), 'Identification of *Bacillus cereus* group species associated with food poisoning outbreaks in British Columbia, Canada', *Appl Environ Micobiol*, 74, 7451–7453.

MCKILLIP J L and DRAKE M (2004), 'Real-time nucleic acid-based detection methods for pathogenic bacteria in food', *J Food Prot*, 67, 823–832.

MÉNDEZ MARTÍNEZ C, PÁEZ JIMÉNEZ A, CORTÉS BLANCO M, SALMORAL CHAMIZO E, MOHEDANO MOHEDANO E, PLATA C, VARO BAENA A and MARTÍNEZ NAVARRO F (2003), 'Brucellosis outbreak due to unpasteurized raw goat cheese in Andalucia (Spain), January–March 2002', *Euro Surveill*, 8, 421.

MILNER M G, SAUNDERS J R and MCCARTHY A J (2001), 'Relationship between nucleic acid ratios and growth in *Listeria monocytogenes*', *Microbiol*, 147, 2689–2696.

MYERS K M, GABA J and AL-KHALDI S F (2006), 'Molecular identification of *Yersinia enterocolitica* isolated from pasteurized whole milk using DNA microarray chip hybridization', *Mol Cell Probes*, 20, 71–80.

NEHA (2008), 'NEHA position regarding the sale or distribution of raw milk', *J Environ Health*, 70, 38–39.

NOCKER A and CAMPER A K (2009), 'Novel approaches toward preferential detection of viable cells using nucleic acid amplification techniques', *FEMS Microbiol Lett*, 291, 137–142.

NOGVA H K, DRØMTORP S M, NISSEN H and RUDI K (2003), 'Ethidium monoazide for DNA-based differentiation of viable and dead bacteria by 5′-nuclease PCR', *Biotechniques*, 34, 804–808, 810, 812–813.

NORTON D M and BATT C A (1999), 'Detection of viable *Listeria monocytogenes* with a 5′ nuclease PCR assay', *Appl Environ Microbiol*, 65, 2122–2127.

O'BRIEN S J, GILLESPIE I A, SIVANESAN M A, ELSON R, HUGHES C and ADAK G K (2006), 'Publication bias in foodborne outbreaks of infectious intestinal disease and its implications for evidence-based food policy. England and Wales 1992–2003', *Epidemiol Infect*, 134, 667–674.

O'GRADY J, SEDANO-BALBÁS S, MAHER M, SMITH T and BERRY T (2008), 'Rapid real-time PCR detection of *Listeria monocytogenes* in enriched food samples based on the *ssrA* gene, a novel diagnostic target', *Food Microbiol*, 25, 75–84.

OLIVEIRA T C, BARBUT S and GRIFFITHS M W (2005), 'A robotic DNA purification protocol and real-time PCR for the detection of *Campylobacter jejuni* in foods', *J Food Prot*, 68, 2131–2135.

OLIVER S P, JAYARAO B M and ALMEIDA R A (2005), 'Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications', *Foodborne Pathog Dis*, 2, 115–129.

OLSEN S J, YING M., DAVIS M F, DEASY J, HOLLAND B, IAMPIETRO L, BAYSINGER C M, SASSANO F, POLK L D, GORMLEY B, HUNG M J, PILOT K, ORSINI M, VANDUYNE S, RANKIN S, GENESE C, BRESNITZ E A, SMUCKER J, MOLL M and SOBEL J (2004), 'Multidrug-resistant

Salmonella Typhimurium infection from milk contaminated after pasteurisation', *Emerg Infect Dis*, 10, 932–935.

OLTES S and YALCIN B (2008), 'Intelligent food packaging', *Log Forum*, 4, 4, 3.

OTTAVIANI F, OTTAVIANI M and AGOSTI M (1997), 'Differential agar medium for *Listeria monocytogenes*', *Quimper Froid, Symposium Proceedings*, pp 6, Quimper (France), A.D.R.I.A.

PALCHETTI I and MASCINI M (2008), 'Electroanalytical biosensors and their potential for food pathogen and toxin detection', *Anal Bioanal Chem*, 391, 455–471.

PERRY J D and FREYDIÈRE A M (2007), The application of chromogenic media in clinical microbiology, *J Appl Microbiol*, 103, 2046–2055.

POLI A, GUGLIELMINI E, SEMBENI S, SPIAZZI M, DELLAGIO F, ROSSI F and TORRIANI S (2007), 'Detection of *Staphylococcus aureus* and enterotoxin genotype diversity in Monte Veronese, a protected designation of origin Italian cheese', *Lett Appl Microbiol*, 45, 529–534.

PRADEL N, BERTIN Y, MARTIN C and LIVRELLI V (2008), 'Molecular analysis of Shiga toxin-producing *Escherichia coli* strains isolated from haemolytic-uremic syndrome patients and dairy samples in France', *Appl Environ Microbiol*, 74, 2118–2128.

QVIST S (2007), 'NordVal: a Nordic system for validation of alternative microbiological methods', *Food Contr*, 18, 113–117.

RAMOS J M, BERNAL E, ESGUEVILLAS T, LOPEZ-GARCIA P, GAZTAMBIDE M S and GUTIERREZ F (2008), 'Non-imported brucellosis outbreak from unpasteurized raw milk in Moroccan immigrants in Spain', *Epidemiol Infect*, 136, 1552–1555.

RASOOLY A and HEROLD K E (2006), 'Biosensors for the analysis of food- and waterborne pathogens and their toxins', *J AOAC Int*, 89, 873–883.

RASOOLY A and HEROLD K E (2008), 'Food microbial pathogen detection and analysis using DNA microarray technologies', *Foodborne Pathog Dis*, 5, 531–550.

REICHERT-SCHWILLINSKY F, PIN C, DZIECIOL M, WAGNER M and HEIN I (2009), 'Stress- and growth rate-related differences between plate count and real-time PCR data during growth of *Listeria monocytogenes*', *Appl Environ Microbiol*, 75, 2132–2138.

RIDER T H, PETROVICK M S, NARGI F E, HARPER J D, SCHWOEBEL E D, MATHEWS R H, BLANCHARD D J, BORTOLIN L T, YOUNG A M, CHEN J and HOLLIS M A (2003), 'A B cell-based sensor for rapid identification of pathogens', *Science*, 301, 213–215.

RIORDAN T, HUMPHREY T J and FOWLES A (1993), 'A point source outbreak of campylobacter infection related to bird-pecked milk', *Epidemiol Infect*, 110, 261–265.

RIPP S, JEGIER P, BIRMELE M, JOHNSON C M, DAUMER K A, GARLAND J L and SAYLER G S (2006), 'Linking bacteriophage infection to quorum sensing signalling and bioluminescent bioreporter monitoring for direct detection of bacterial agents', *J Appl Microbiol*, 100, 488–499.

RUDOL M and SCHERER S (2001), 'High incidence of *Listeria monocytogenes* in European red smear cheese', *Int J Food Microbiol*, 63, 91–98.

RYAN C A, NICKELS M K, HARGRETT-BEAN N T, POTTER M E, ENDO T, MAYER L, LANGKOP C W, GIBSON C, MCDONALD R C and KENNEY R T (1987), 'Massive outbreak of antimicrobial-resistant salmonellosis traced to pasteurized milk', *JAMA*, 258, 3269–3274.

RYSER E T and DONNELLY C W (2001), 'Listeria', Chapter 36 in Downes F P *et al*., *Compendium of Methods for the Microbiological Examination of Foods*, Washington, DC, American Public Health Association.

SCHILDT M, SAVOLAINEN S and HÄNNINEN M L (2006), 'Long-lasting *Campylobacter jejuni* contamination of milk associated with gastrointestinal illness in a farming family',

Epidemiol Infect, 134, 401–405.

SCHLUNDT J, TOYOFUKU H, JANSEN J and HERBST S A (2004), 'Emerging food-borne zoonoses', *Rev Sci Tech*, 23, 513–533.

SCHODER D, WINTER P, KAREEM A, BAUMGARTNER W and WAGNER M (2003), 'A case of sporadic ovine mastitis caused by *Listeria monocytogenes* and its effect on contamination of raw milk and raw-milk cheese produced in the on-farm dairy', *J Dairy Res*, 70, 395–401.

SINGH J, BATISH V K and GROVER S (2009), 'A scorpion probe-based real-time PCR assay for detection of *E. coli* O157:H7 in dairy products', *Foodborne Pathog Dis*, 6, 1–6.

SOEJIMA T, NAGAO E, YANO Y, YAMAGATA H, KAGI H and SHINAGAWA K (2007), 'Risk evaluation for staphylococcal food poisoning in processed milk produced with skim milk powder', *Int J Food Microbiol*, 115, 29–34.

SOLER P, HERRERA S, RODRÍGUEZ J, CASCANTE J, CABRAL R, ECHEITA-SARRIONDIA A and MATEO S (2008), 'Nationwide outbreak of *Salmonella enterica* serotype Kedougou infection in infants linked to infant formula milk, Spain, 2008', *Euro Surveill*, 13, pii=18963.

SPREER E (1998),'Milk as a raw material and food', in Spreer E, *Milk and Dairy Product Technology*, New York, Marcel Dekker, 39–41.

STEVENS K A and JAYKUS L A (2004), 'Bacterial separation and concentration from complex sample matrices: a review', *Crit Rev Microbiol*, 30, 7–24.

STRATMANN J, DOHMANN K, HEINZMANN J and GERLACH G F (2006), 'Peptide aMptD-mediated capture PCR for detection of *Mycobacterium avium* subsp *paratuberculosis* in bulk milk samples', *Appl Environ Microbiol*, 72, 5150–5158.

STUART J, SUFI F, MCNULTY C and PARK P (1997), 'Outbreak of campylobacter enteritis in a residential school associated with bird pecked bottle tops', *Common Dis Rep CDR*, 7, R38–40.

SVENSSON B, ENEROTH Å, BRENDEHAUG J, MOLIN G and CHRISTIANSSON A (2000), 'Involvement of a pasteurizer in the contamination of milk by *Bacillus cereus* in a commercial dairy plant', *J Dairy Res*, 67, 455–460.

SWAMINATHAN B and GERNER-SCHMIDT P (2007), 'The epidemiology of human listeriosis', *Microbes Infect*, 9, 1236–1243.

TABOADA E N, VAN BELKUM A, YUKI N, ACEDILLO R R, GODSCHALK P C, KOGA M, ENDTZ H P, GILBERT M and NASH J H (2007), 'Comparative genomic analysis of *Campylobacter jejuni* associated with Guillain–Barré and Miller Fisher syndromes: neuropathogenic and enteritis-associated isolates can share high levels of genomic similarity', *BMC Genomics*, 8, 359.

TODD E C, GREIG J D, BARTLESON C A and MICHAELS B S (2008), 'Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 4. Infective doses and pathogen carriage', *J Food Prot*, 71, 2339–2373.

TOMBELLI S, MINUNNI M and MASCINI M (2007), 'Aptamers-based assays for diagnostics, environmental and food analysis', *Biomol Eng*, 24, 191–200.

US FDA (2001), *Bacteriological Analytical Manual*, Silver Spring, MD, Food and Drug Administration. Available from http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm [accessed 4 July 2009].

US FDA (2005), *Compliance Policy Guide, Pathogens in Dairy Products Sec. 527.300*, Silver Spring, MD, Food and Drug Administration. Available from http://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074480.htm [accessed 9 July 2009].

US FDA (2007), PMO 2007, *Standards for Grade 'A' Raw Milk – Pasteurization, Ultra-Pasteurization, Aseptic Processing*, Silver Spring, MD, Food and Drug Administration. Available from http://www.fda.gov/Food/FoodSafety/Product-Specific Information/MilkSafety/NationalConferenceonInterstateMilk ShipmentsNCIMS ModelDocuments/PasteurizedMilkOrdinance2007/ucm063931.htm [accessed 6 July 2009].

US FDA (2008), *US FDA/CFSAN – Bad Bug Book*, Silver Spring, MD, Food and Drug Administration. Available from http://vm.cfsan.fda.gov/~mow/intro.html [accessed 4 July 2009].

VANACKER J, DESMET F, MUYLDERMANS G, BOUGATEF A, NAESSENS A and LAUWERS S (2001), 'Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula', *J Clin Microbiol*, 39, 293–297.

VILLAR R G, MACEK M D, SIMONS S, HAYES P S, GOLDOFT M J, LEWIS J H, ROWAN L L, HURSH D, PATNODE M and MEAD P S (1999), 'Investigation of multidrug-resistant *Salmonella* serotype Typhimurium DT104 infections linked to raw-milk cheese in Washington state', *JAMA*, 281, 1811–1816.

WEHR M (2004), 'Standard methods', Chapter 1 in Wehr M and Frank J F, *Standard Methods for the Examination of Dairy Products*, Washington, DC, American Public Health Association.

WHITMAN D F and DUNBAR S A (2008), 'Real-time polymerase chain reaction detection methods', *Recent Pat DNA Gene Seq*, 2, 20–26.

YANG H, QU L, WIMBROW A N, JIANG X and SUN Y (2007), 'Rapid detection of *Listeria monocytogenes* by nanoparticle-based immunomagnetic separation and real-time PCR', *Int J Food Microbiol*, 118, 132–138.

5

Pesticides, veterinary residues and other contaminants in milk

S. K. Nag, Indian Grassland and Fodder Research Institute, India

Abstract: This chapter deals with the various chemical contaminants such as pesticide residues, heavy metals and radionuclides, veterinary drugs and antibiotics, mycotoxins, nitrates and nitrites, detergents and disinfectants present in milk and milk products. Apart from reviewing the extent and level of contamination through these chemicals in different places and countries, this chapter also discusses the health impacts of the contaminants, various sources of contamination, analytical techniques, regulatory aspects and methods to manage the contaminants.

Key words: contaminants, milk, milk products, pesticide residues, heavy metals, radionuclides, aflatoxins, mycotoxins, veterinary drugs, antibiotics, nitrates, detergents, disinfectants.

5.1   Introduction

Milk is considered to be the most balanced food ever found in nature containing most of the nutrients. It is consumed by people of all ages and nationalities, although the amount may vary according to food habit and availability. However, it is mostly required for infants, children, the aged and the sick. Milk is the only source of animal protein in the diet of many vegetarians, whose numbers are increasing across the globe. Milk is also regarded as a sacred food for people with particular religious beliefs. Besides raw milk, various products made from

it, such as butter, ghee, cheese, paneer, yoghurt, sweetmeats, ice creams etc., are produced and consumed by people all over the world.

However, milk of animals is exposed to and gets contaminated with diverse chemical substances at various stages during its production and storage. Milk is produced inside the body by a physiological process incorporating biochemical conversion of nutrients derived from various dietary constituents. During the process it is exposed to many chemical contaminants carried by the feed, fodder and other dietary constituents including water.

The presence of any substances in food such as xenobiotics, having toxic effects, is undesirable, since it has a direct link to the health of consumers. This is more so in the case of milk because milk plays a very important role in the diet of infants and children. Thus, chemical contaminants present in milk and its products may cause physical illness if a significant amount is consumed, as would be the case for those who consume milk on a regular basis.

The various types of chemical contaminants which may be found in milk are:

• Pesticide residues
• Other POPs (persistent organic pollutants) such as PCBs, PCDDs, PCDFs, etc.
• Heavy metals
• Radionuclides
• Veterinary drugs and antibiotics
• Aflatoxins and mycotoxins
• Nitrites and nitrates
• Detergents and disinfectants.

5.2 Pesticide residues and other chemical contaminants in milk and their potential impact on health

5.2.1 Pesticide residues

Pesticides are any substances or mixtures intended for preventing, controlling or destroying any pest organisms or unwanted species of plants or animals, which have the potential to cause damage at any stage during production and storage of agricultural commodities or adversely impact on the health of livestock and human beings. Whenever synthetic pesticides, known as toxic xenobiotics, are applied on any substrate for controlling or managing the target pests, then apart from exerting their desired action they invariably leave residues, the persistence of which depends on many factors like the nature and type of the molecule, rate and volume of application, nature of the substrate on which they are applied, surrounding environmental conditions and so on. Residues of pesticides are also found in places or substances where they had not been used directly but had appeared to have come from distant places or materials where they had been used earlier, through volatilisation or cross-contamination through translocation.

Dairy milk and its products have been contaminated with residues of pesticides, particularly organochlorines (OCs), which has been a matter of great

concern because of the special significance of these food items in the diet of infants and children. The reason for the existence of pesticide residues in milk or dairy products is not due to any direct application but is due mainly to transfer from contaminated feed and fodder offered to animals for consumption.

Organochlorines as a class are regarded as having low acute toxicity but possess a greater potential for chronic toxicity when compared to other classes, i.e., organophosphates, carbamates, synthetic pyrethroids and others. Organochlorinated pesticides (OCPs), particularly DDT and HCH along with cyclodienes like aldrin, dieldrin, chlordane and heptachlor, have been encountered in milk and its products over the past few decades. OC pesticides, designated as the most persistent, have been extensively used throughout the world, particularly in the tropics, in order to boost agricultural production by controlling insect pests and in human and animal health applications to control the vectors causing diseases like malaria and others. During the global malaria control programme thousands of tonnes of DDT were used for vector control from the 1950s to the 1970s. After that their use was discontinued in developed countries but still continued in developing countries of Asia, Africa, Central and South America. The unusual and high persistence of the OCPs is due to their chemical inertness, stability and lipophilic character. Among the different OCs, DDTs and HCBs are very highly lipophilic while HCHs are comparatively less lipophilic. Their very high lipid solubility allows the OCPs to accumulate in the tissues of the body. After entering the body they enter a steady state and bioconcentrate in tissue lipids, according to equilibrium patterns of internal transport and lipid tissue content. The lipid-rich tissues act as depots or reservoirs of persistent OCs by virtue of their physico-chemical interactions with cellular components, and their concentrations decline at a very slow rate, even after sources of contamination are eliminated.

OCPs display various types and degrees of toxicity. Residues accumulate in fatty tissues, thus building up in the vital organs such as thyroid, heart, kidney, liver, mammary gland and testes. Several health effects ranging from systemic cardiovascular and respiratory effects to genotoxicity have been reported (Kalpana, 1999). These residues can also be transferred from the umbilical cord to the foetus and through breast-feeding to babies. Researchers have linked exposure to them with increased risk of cancer in humans and some of these pesticides are carcinogenic in animal test systems. Many of the OCPs are now recognised as potential endocrine disruptors in humans, even at low levels of exposure (Colborn *et al.*, 1993; Kalpana, 1999).

Because of their vapour pressures and partitioning behaviour under ambient conditions, persistent OCPs are mobile in the environment and thus bioaccumulate in the environment and consequently in the food chain. Therefore, they have the propensity for long-range atmospheric transport and to undergo global-scale redistribution where they condense and accumulate in colder regions. Studies on bioaccumulation potential and hazards of OCs on human and animal life have shown that the principal mode of transport of OCs is via the atmosphere, which accounts for the ubiquitous presence of OCs throughout the

world, including the Arctic and Antarctic ecosystems. In the tropical agro-ecosystem, which is characterised by high temperatures and heavy rainfall, semi-volatile organic compounds are rapidly dissipated. Vitalised residues from the tropics disperse through the atmosphere and OCs are ultimately deposited in cold and temperate regions as a result of atmospheric fallout.

The significance of pesticide contamination in milk has been viewed with great importance since the 1960s. All the monitoring studies carried out on milk for pesticide residues focused on OCPs for obvious reasons and the data revealed mostly the presence of DDTs and HCHs, many times in very alarming concentrations, with others such as aldrin, dieldrin, etc., being observed in a few instances.

DDT residues

Surveys conducted since 1965 have shown residues of DDT in bovine milk often exceeding the MRL (Maximum Residue Limit) of 1.25 mg/kg (fat basis) or 0.05 mg/kg (whole milk basis) as prescribed by FAO/WHO. Tripathi (1966) found four out of five milk samples from Pantnagar, India, containing DDT residues but below the MRL level. From Delhi 13 samples out of 17 in 1965 were contaminated with DDT residues and concentrations exceeded the MRL in nine samples. But in 1972, 13 Delhi milk samples from a batch of 14 were positive for DDT at a concentration much higher than the MRL (Agnihotri *et al.*, 1974). The maximum concentration of DDT in milk collected from Delhi and Hyderabad in 1972 was 2 and 5 mg/kg, respectively (Kannan *et al.*, 1997). DDT concentration in milk from Punjab, India, during 1979–1981 was 0.05–1.57 mg/kg (Singh *et al.*, 1986), while milk from Bombay contained DDT up to 10 mg/kg, averaging 5 mg/kg (Khandekar *et al.*, 1981).

Although many earlier reports mentioned only the presence of residues of the DDT molecule in milk, it is now accepted that DDT residues should be measured and represented as DDT isomers (op[l] and pp[l]), as well as its metabolites, viz. isomers of DDD and DDE. The total DDT refers to the sum of all these components present in a single sample. The ratio of pp[l] DDT to total DDT, or of DDT to DDE, gives an indication of how recently DDT formulations have been released into the environment, with the ratio decreasing over time as the DDT degrades. High levels of DDE in milk may originate not only from the previous use of DDT but also from the use of dicofol or kelthane, a non-systemic acaricide used in many places. According to Brown *et al.* (1986), α-chloro-DDT, a dicofol impurity, occurs at levels as high as 10% and undergoes facile photochemical dechlorination, thereby providing a probable source for environmental DDE.

Dhaliwal and Kalra (1977) found DDT residues in milk supplied by the Punjab Dairy Development Corporation having a mean concentration of 0.26 mg/kg, 72% of which was constituted by pp[l] DDD followed by pp[l] DDT and pp[l] DDE. In a four state survey conducted by FAO and the Indian government during the mid-1980s, DDT was found in 95% of samples with a range of 0.19–216 mg/kg on a fat basis (Kalra and Chawla, 1985). In one report from

Maharashtra, buffalo and cow milk was found to contain residues at levels of 0.014–1.75 mg/kg and 0.003–1.42 mg/kg, respectively (Jadhav, 1986). In Uttar Pradesh, India, levels of DDT of 0.22 mg/kg were recorded in milk in the mid-1980s. According to a report from the All India Co-ordinated Research Project (AICRP) on pesticide residues, ICAR, New Delhi, out of 487 milk samples collected from across India and analysed for pesticide residues, DDT was detected in 86.5% of samples, of which 43.2% contained residues above the prescribed MRL of 0.05 mg/kg. High levels of DDT residues were detected in samples from New Delhi (0.01–1.08 mg/kg), Anand (0.18–25.6 mg/kg), Ludhiana (0.01–0.28 mg/kg), Hyderabad (not detected (ND)–0.25 mg/kg), Rahuri (ND–0.53 mg/kg), Pusa (ND–0.17 mg/kg) and Coimbatore (0.01–0.43 mg/kg) (Agnihotri, 1999). DDT residue in excess of its MRL was found in all dairy samples taken from in and around Delhi in 1992–93 by Mukherjee and Gopal (1993). However, only 10–20% of bovine milk samples contained DDT above the MRL in samples from the Hisar, Himachal Pradesh and Karaikal regions of India (Kathpal *et al.*, 1992; Kumar and Nath, 1996; Adiroubane and Letchoumanae, 1996).

In a multi-centre study conducted by ICMR (Indian Council of Medical Research) in 12 Indian states, 82% of 2205 dairy milk samples were found to have DDT residues above the detection level and 37% were above the MRL level (Table 5.1) (Kalra *et al.*, 1999). In 2001, Nigam and Siddiqui (2001) reported 100% contamination with DDT in milk collected from two dairies in Lucknow (India) with the range of concentration being 0.01–0.03 mg/kg. Different branded and unbranded milk from various cities of Maharashtra

Table 5.1 Extent of contamination of dairy milk samples with DDT residues in different states of India

State	No. of samples analysed	No. of samples above detection limit	No. of samples with residues above MRL	Mean conc. (mg/kg, whole milk basis)
Himachal Pradesh	120	120 (100%)	67 (55.8%)	0.091
Punjab	263	257 (97.7%)	133 (50.6%)	0.111
Haryana	120	116 (96.7%)	6 (5.0%)	0.022
Uttar Pradesh	240	137 (57.1%)	26 (10.8%)	0.030
Madhya Pradesh	240	230 (95.8%)	52 (21.7%)	0.042
Maharashtra	299	299 (100%)	222 (74.2%)	0.080
Gujarat	120	120 (100%)	84 (70.0%)	0.091
West Bengal	120	43 (35.8%)	15 (12.5%)	0.021
Bihar	120	115 (95.8%)	23 (19.2%)	0.041
Andhra Pradesh	240	208 (86.7%)	137 (57.1%)	0.207
Karnataka	203	45 (22.2%)	36 (17.7%)	0.047
Kerala	120	115 (95.8%)	11 (9.2%)	0.030
Combined	2205	1805 (81.9%)	812 (36.8%)	0.075

Source: Kalra *et al.* (1999).

contained DDT residues varying from 0.016 to 0.338 mg/kg (fat basis) in 1999 but none exceeded the FAO/WHO tolerance level (Pandit *et al.*, 2002). Analysis of bovine milk samples collected from the Tamilnadu Agricultural University (TNAU) dairy farm and private vendors showed 24% contamination with DDT but at below the MRL (Vasanthi *et al.*, 2003). DDT contamination was present in only six samples (6.52%) out of 92 liquid milk samples obtained from Ludhiana district of Punjab during 1999–2001 (Battu *et al.*, 2004). Of these, two samples exceeded the MRL and this was contrary to the earlier report by this research group (Battu *et al.*, 1996), which found that 92% of milk samples from the same district exceeded the MRL for DDT. These results clearly indicate a change in the contamination of liquid milk with DDT residues over time.

Kumar *et al.* (2005) found pp[l] DDE as the major constituent with a mean value of 0.055 mg/kg, followed by pp[l] DDT (0.04 mg/kg) and op[l] DDT (0.01 mg/kg), as the composition of total DDT residues found in milks from Agra, India. The mean level of DDT in milk samples collected from southern states of India during 1999–2002 was 0.12 mg/kg as against 0.41 mg/kg in samples taken during 1992–93 (Surendra Nath *et al.*, 2005), which again depicts a decline in the load of DDT in milk, and this was similar to earlier reports (Unnikrishnan *et al.*, 1999). DDE was found to be the major constituent of the total DDT content in milks sampled between 1999 and 2002.

A report published in 2007 (Sharma *et al.*, 2007) showed that all the 140 bovine milk samples taken from different districts of Haryana during December 1998 to February 1999 contained DDT with a mean value of 0.0292 mg/kg. Of 325 bovine milk samples of the Bundelkhand region of India, 114 (35.07%) were contaminated with DDT (traces to 0.98 mg/kg) and the MRL was exceeded in 38 samples. The major component was pp[l] DDE followed by op[l] DDE, pp[l] DDD, pp[l] and op[l] DDT (Nag and Raikwar, 2008).

The above reports indicate widespread contamination of milk with DDT residues across different states of India, particularly during 1970–2000. In contrast, the residue levels in milk and milk products in developed nations were much less. The DDT contamination level in cow milk in Japan was well below the MRL level (Uyeta *et al.*, 1970). The highest levels of 0.08 mg/kg of total DDT were reported from Miyagi and Okayama (Tanabe, 1972; Tomizawa, 1977). The cow milk in three Central American countries, i.e. El Salvador, Guatemala and Honduras, contained DDT residues varying from 0.3 to 32.31 mg/kg with an average of 4.22 mg/kg (Mazariegos, 1976). The results obtained from the analysis of cow milk samples taken from 12 commercial dairies in Israel revealed an average of 0.29 mg/kg of total DDT on a fat basis (Veierov *et al.*, 1977). In a preliminary study of organochlorine compounds in Greek milk products, DDT residue was almost absent in 30 milk samples collected from different areas of Northern Greece (Fytianos *et al.*, 1985). The majority of cow milk samples acquired at random in 1990 and 1991 in different markets in Madrid (Spain) contained pp[l] DDE with a mean concentration of 13.9 ng/g (Hernandez *et al.*, 1994). A survey of 252 milks from a Hong Kong market between 1993 and 1995 showed DDE contamination in 73% of samples with a

mean level of 0.16 mg/kg, but only 8% and 5% of samples contained DDD and DDT with mean concentrations of 0.14 and 0.05 mg/kg, respectively (Wong and Lee, 1997).

The results of monitoring 192 cow milk samples from the Veracruz state of Mexico revealed that 46% samples were contaminated with pp[l] DDE, with average concentration of 0.028 mg/kg, while only 27% contained pp[l] DDT ranging from 0.01–0.603 mg/kg with a mean of 0.078 mg/kg (Waliszewski *et al.*, 1996). Again analysis of 150 cow milks, taken in 1998 and 2001 from the city of Veracruz, revealed the presence of pp[l] DDT (mean value 0.078 and 0.037 mg/kg, respectively) and pp[l] DDE (mean value 0.051 and 0.033 mg/kg, respectively). The op[l] DDT was detected in lower quantities (0.008–0.06 mg/kg) and it was also noted that when compared with previous years DDT levels had declined significantly over the years (Waliszewski *et al.*, 2003). A 2001 survey of organochlorine pesticides in retail milk from Beijing showed that among the DDT isomers pp[l] DDE was the main form detected, because most DDT in the environment has been degraded to the stable DDE form. Out of 72 milks, 21 were contaminated with DDT, with average concentration of 0.046 mg/kg (Zhong *et al.*, 2003). A monitoring programme of cow milk from tropical regions of Mexico (Medellin, Paso San Juan and Tlalixcoyan) demonstrated 70–100% contamination with pp[l] DDE and 43–89% with pp[l] DDT, with mean concentrations of 0.018–0.039 and 0.036–0.089 mg/kg, respectively. However, pp[l] DDD could not be detected in any sample, while op[l] DDT was found only in samples from Medellin (Pardio *et al.*, 2003).

HCH residues

HCH (hexachlorocyclohexane) is also known as BHC (benzene hexachloride), although this is a misnomer. The residues of HCH in any product or substrate are estimated on the basis of its four major isomers, viz., α, β, γ and δ. Total HCH refers to the summation of all these four isomers present in a single sample. Technical or commercial HCH consists of 55–70% α-HCH, 5–14% β-HCH, 10–18% γ-HCH, 6–18% δ-HCH and 3–4% η and ϵ-HCH. Of these the β and ϵ isomers are inactive, the α, δ and η isomers are slightly to moderately active, and the γ isomer is the most active. The pure form of γ-HCH, known as lindane, is also used commercially (95% γ isomer). It is 1000 times more active than HCH. The isomers other than lindane, although non-insecticidal, are considered to be hazardous to human health. The acceptable daily intakes (ADI) of α-HCH, β-HCH and γ-HCH are 0.005, 0.001 and 0.005 mg/kg/day, respectively. HCH is reported to be a carcinogen.

Contamination of bovine milk has been found to be widespread and excessive as reported from many countries during the last few decades. Lakshminarayana and Menon (1975) found 25% of bovine milks to be contaminated with HCH residues, ranging in concentration from traces to 5 mg/kg. All the 54 samples collected from rural areas of Punjab contained HCH with the level of contamination ranging from 0.014 to 2.067 mg/kg (Kapoor *et al.*, 1980). Almost 90% of a total of 980 samples of milk from the states of Andhra Pradesh,

Maharashtra, Delhi and Punjab (India) contained HCH residues at levels ranging from 0.12 to 0.40 mg/kg on a fat basis (Kalra and Chawla, 1981). Elevated levels of HCH over those of DDT in milk from paddy-growing areas in Tamilnadu in 1990 have been found. The average residue levels in cow and buffalo milk were 0.11 (0.08–0.18) and 0.25 (0.13–0.49) mg/kg, respectively (Kannan *et al.*, 1992). All the 54 bovine milk samples collected from Hisar (Haryana, India) contained HCH, having a mean concentration of 0.078 mg/kg and comprising α, β, γ and δ isomers (Kathpal *et al.*, 1992). About 26% of a total of 50 bovine milk samples from different agroclimatic zones of Himachal Pradesh, India, were found to contain γ-HCH residues above its MRL, while the total HCH varied from 0.053 to 0.419 mg/kg (Kumar and Nath, 1996).

Again, all of the 21 bovine milk samples from Karaikal region, in the Union Territory of Pondicherry, India, were found to be contaminated with HCH residues (0.028–0.14 mg/kg) comprising the α, β and γ isomers (Adiroubane and Letchoumanae, 1996). Nine bovine milk samples collected from Jaipur (India) showed the presence of HCH residues, of which five samples had a β-HCH level above its MRL (Gupta *et al.*, 1997). A multi-centre study conducted by ICMR on 2205 milk samples collected from rural and urban areas representing different geographical regions of India revealed contamination with α-HCH (87%), β-HCH (85%), γ-HCH (55%) and δ-HCH (51%) and the concentrations exceeded the respective MRLs in 21%, 42%, 28% and 4% of samples, respectively (Kalra *et al.*, 1999). Samples collected from two dairies in Lucknow were 100% positive for HCH residues. The α, β and γ isomers were present at concentrations above the MRL in 60–70%, 80–100% and 70–90% of samples, respectively, while none exceeded the MRL in the case of δ-HCH (Nigam and Siddiqui, 2001). A comparison of these data with those generated previously in Lucknow samples (Saxena and Siddiqui, 1982) suggested that there had been a greater than three-fold increase in HCH levels in milk, although there was a 50% decline in the prevalence of milk containing DDT residues.

All the branded and unbranded milk samples from different cities of Maharashtra collected during 1999 showed the presence of α-, β- and γ-HCH residues. The β-HCH contributed nearly 30% to the total HCH, which ranged from 0.009 to 0.169 mg/kg, and the ratio of α to γ HCH varied from 0.7 to 1.6, which suggested that most of the HCH came from the use of technical-grade HCH rather than lindane (Pandit *et al.*, 2002). Out of 69 bovine milks collected from the dairy farm of TNAU and private vendors, 84% were contaminated with HCH residues (Vasanthi *et al.*, 2003). About 53% (49 out of 92 samples) taken from Ludhiana (India) during 1999–2000 contained HCH residues in the form of only the γ isomer, which was present at levels above the MRL in all the contaminated samples (Battu *et al.*, 2004). In the city of Agra (India) testing of milk collected from 65 dairies revealed the presence of HCH (α, β and γ) residues in all the samples. The mean concentration of total HCH was 0.114 mg/kg and the concentration of β and γ isomers (0.048 mg/kg) was more than that of the α isomer (0.018 mg/kg) (Kumar *et al.*, 2005). One hundred percent contamination with HCH residues (mean concentration 0.0292 mg/kg) was also recorded

in 147 samples of bovine milk collected from 14 districts of Haryana during 1998–99. Eighty percent of the milks exceeded the MRL of 0.1 mg/kg as recommended by WHO for total HCH, and 4%, 5% and 26% of samples exceeded the MRL recommended by PFA (Prevention of Food Adulteration Act, Government of India) for α-HCH (0.05 mg/kg), γ-HCH (0.01 mg/kg) and β-HCH (0.02 mg/kg), respectively. The concentration of β-HCH was more than that of the other isomers of HCH (Sharma *et al.*, 2007). Out of 325 bovine milk samples taken during 2002–05 from the Bundelkhand region of India, HCH residues could be detected in 123 samples. The total HCH varied from traces to 0.92 mg/kg, with an average value of 0.162 mg/kg. The β isomer was found at levels above the MRL in 55% of the contaminated samples, followed by the γ (29%), δ (25%) and α isomers (6%) (Nag and Raikwar, 2008).

Cow's milk in Japan was found to be severely contaminated with HCH in 1969. The prevalence of β-HCH constituted 66.8% followed by the α, δ and γ isomers at 26.9, 4.3 and 2.0%, respectively (Uyeta *et al.*, 1970). The higher levels of β-HCH seem to be due to greater stability, lower volatility and higher capacity to accumulate in lipids. Milk samples collected from 12 commercial dairies in Israel revealed the highest contamination of 0.037 mg/kg for α-HCH followed by 0.28 mg/kg for γ-HCH (Veierov *et al.*, 1977). The β isomer was found at levels of less than 0.05 mg/kg. In West Germany it was also found that quite a high lindane concentration (up to 1.9 mg/kg) existed in cow milk samples (Koch and Varenholt, 1981).

Analysis of 240 milk samples from the central tropical region of Mexico (Veracruz) showed 44–100% detection frequency of HCH isomers with a mean concentration of total HCH varying between 0.053 and 0.230 mg/kg. The samples from the Tlalixcoyan area showed 100% contamination with α-, β- and γ-HCH having average concentrations of 0.031, 0.069 and 0.128 mg/kg, respectively, which was significantly higher than that in samples from other locations such as Medellin and Paso San Juan (Pardio *et al.*, 2003). In a previous study of the same region but from different dairies, α-, β- and γ-HCH were detected in milk samples with mean levels of 0.055, 0.095 and 0.026 mg/kg, respectively (Waliszewski *et al.*, 1996). The α- and β-HCH levels were higher than those detected in Tlalixcoyan samples as reported by Pardio *et al.* (2003); nevertheless, the γ-HCH level was five times lower. The results indicated a greater usage of HCH in this region, which was a concern because the other isomers can metabolically transform to the stable isomer β-HCH, which is predominantly accumulated in human and animal adipose tissue (Kalantzi *et al.*, 2001). Environmental β-HCH originates from lindane which isomerises in the environment to α- and β-HCH (Steinwandter, 1978; Steinwandter and Schlüter, 1978; Waliszewski, 1993a,b, 1995). The level of β-HCH (0.069 mg/kg) detected in milk from Tlalixcoyan was similar to those found in India but higher than the levels detected in Spain (Hernandez *et al.*, 1994) and Slovakia (Prachar *et al.*, 1995).

Owing to its persistence, rapid bio-concentration of β-HCH takes place in humans. The bio-concentration potential of β-HCH is higher and elimination is

slower than for the other HCH isomers (WHO, 1992; Sang *et al.*, 1999). Because of its high lipid solubility HCH is more deposited in fat but less in liver, kidney and brain and it is also excreted in milk. Among the different isomers, the transfer coefficient, defined as 'the percentage of daily intake of pesticide which is excreted in the milk each day after equilibrium conditions have been reached', is highest in the case of β-HCH (31–36%) followed by α-HCH (12–15%), δ-HCH (8.5%) and γ-HCH (2.7%). For DDT the transfer coefficient is 5%.

Out of 72 milk samples collected during 2001 from a supermarket in Beijing, China, nine to 36 samples were found to be contaminated with four HCH isomers (α, β, γ and δ) and the mean concentration of total HCH was 0.038 mg/kg on a fat basis. The concentration of the most important γ-isomer was between 0.003 and 0.35 mg/kg on a fat basis, and the frequency of occurrence of the four isomers was in the order $\alpha > \beta > \gamma > \delta$, while in terms of concentration the sequence was $\alpha > \gamma > \beta > \delta$ (Zhong *et al.*, 2003).

The frequency of β-HCH in cow milk samples taken from the city of Veracruz, Mexico, was 97% and 93% during 1998 and 2001, respectively, indicating widespread contamination by this isomer of HCH. The mean residue levels of 0.106 mg/kg in 1998 and 0.087 mg/kg in 2001 were the highest among all the OC pesticides detected in cow milk (Waliszewski *et al.*, 2003). Alwai and Al-Hawudi (2005) evaluated 60 sheep milk samples for the presence of pesticide residues gathered from different towns in Jordan and found all the milk to be contaminated with HCH residues.

Endosulfan residues
Reports of the occurrence of endosulfan residues in milk are very rare, unlike the DDTs, HCHs and some other OCs. There are a few reasons for this. Firstly, endosulfan came onto the market after the DDTs and HCHs. Secondly, the use of endosulfan is very much restricted only to the field of agriculture, unlike DDTs and HCHs which, apart from agricultural use, have been and still are being used widely as part of public health programmes to combat malaria and some other diseases and also in veterinary healthcare to control animal ectoparasites. Thirdly, but most importantly, endosulfan is not as persistent as the other OCs because it metabolises at a much faster rate to water-soluble metabolites and has a lower partition coefficient. Fourthly, endosulfan has a very much lower transfer coefficient compared to DDTs, HCHs and other OCs. The transfer coefficient of endosulfan has been calculated to be 0.23–0.33% in goat milk (Nag *et al.*, 2007). It has even been reported that endosulfan does not pass into the milk of cattle when ingested in feed for a prolonged period of time (Li *et al.*, 1970; Surendra Nath *et al.*, 2000).

Technically, endosulfan exists as a combination of two stereoisomers, viz. α and β in the ratio of 70:30. Residues of endosulfan are estimated in terms of α-endosulfan, β-endosulfan and its toxic metabolite endosulfan sulfate. Among the two stereoisomers, the β isomer is reported to be more persistent than its α counterpart as the latter partly isomerises to the β isomer and also converts into sulfate at a faster rate in different substrates. Total endosulfan residue present in

Table 5.2 Comparison of DDT and HCH residue levels (mg/kg) in dairy milk from different states of India

State	Year	DDT	HCH	Reference
Himachal Pradesh	1997	0.091	0.037	Kalra *et al.* (1999)
Haryana	1997	0.022	0.051	Kalra *et al.* (1999)
Madhya Pradesh	1997	0.042	0.056	Kalra *et al.* (1999)
West Bengal	1997	0.021	0.170	Kalra *et al.* (1999)
Bihar	1997	0.041	0.179	Kalra *et al.* (1999)
Tamilnadu	1989	0.02	0.18	Kannan *et al.* (1992)
Kerala	1997	0.03	0.082	Kalra *et al.* (1999)
Delhi	1992–93	0.017	0.09	Mukherjee and Gopal (1993)
Andhra Pradesh	1997	0.207	0.563	Kalra *et al.* (1999)
Punjab	1997	0.111	0.067	Kalra *et al.* (1999)
Uttar Pradesh	1997	0.03	0.234	Kalra *et al.* (1999)
Maharashtra	1997	0.080	0.049	Kalra *et al.* (1999)
Gujarat	1997	0.091	0.073	Kalra *et al.* (1999)
Karnataka	1997	0.047	0.179	Kalra *et al.* (1999)

a particular sample is thus calculated as the summation of the three components. Chauhan *et al.* (1982) detected endosulfan in only three of 105 milk samples collected from Hisar (India), the level ranging from traces to 2.5 mg/kg. Endosulfan isomers and its sulfate (0.001–0.154 mg/kg) were recorded in 29 samples above the MRL, out of 155 of bovine milk, again from Hisar (Kathpal *et al.*, 2004). In another monitoring study with 147 milk samples taken from different districts of Haryana, α- and β-endosulfan were detected in 7% and 44% of samples, respectively, with concentrations varying from BDL (below detectable level) to 0.0079 and from BDL to 0.028 μg/ml, respectively (Sharma *et al.*, 2007). Out of 325 bovine milks from the Bundelkhand region of India, α-endosulfan, β-endosulfan and endosulfan sulfate were detected in 77 (traces to 0.036 mg/kg), 62 (0.001–0.315 mg/kg) and 51 samples (traces to 0.131 mg/kg), respectively (Nag and Raikwar, 2008). A comparison of OC pesticide residue levels in milk from different states of India and from different countries is depicted in Tables 5.2 and 5.3.

Other OCPs and POPs
Apart from DDTs, HCHs and endosulfans, other OCPs such as aldrin, dieldrin, chlordane, endrin, heptachlor, etc., have also been detected in milk on many occasions. Milks from Lucknow were found to be contaminated with aldrin at an average level of 0.02 mg/kg in buffalo and 0.005 mg/kg in goat milk (Saxena and Siddiqui, 1982). Khandekar *et al.* (1981) found 12 out of 23 milk samples collected from local vendors in Bombay had alarming levels of dieldrin which varied from 38 to 126 mg/kg on a fat basis. This level was more than 500 times higher than the MRL of 0.05 mg/kg as recommended by FAO/WHO (1979). Aldrin, dieldrin and heptachlor were detected in significant amounts in the samples of milk collected from Andhra Pradesh, India (Kannan *et al.*, 1992).

Table 5.3 Comparison of OC levels (mg/kg) in milk from different countries

Country	Reference	α-HCH	β-HCH	γ-HCH	δ-HCH	Σ-HCH	Σ-DDT	Σ-endosulfan
France	Venant *et al.* (1991)	0.003	0.005	0.024				
Spain	Riva and Anadan (1991)	0.018	0.009	0.024				
China	Zhang (1995)					0.07	0.095	
Mexico	Waliszewski *et al.* (1996)	0.055	0.099	0.026		0.098	0.057	
India (Delhi)	Mukherjee and Gopal (1993)	0.053	0.014	0.004		0.071	0.150	
Slovakia	Prachar *et al.* (1995)	0.005	0.006	0.004		0.015	0.413	
India (all)	Agnihotri (1999)					ND–5.12[a]	ND–25.6[a]	
India (Lucknow)	Nigam and Siddiqui (2001)		0.078	0.025			0.015	
India (south)	Surendra Nath *et al.* (2005)					0.01–0.71	ND–0.8[a]	
China	Zhong *et al.* (2003)	0.024	0.011	0.012			0.046	
India (north)	Kathpal *et al.* (2004)					0.001–0.209	0.001–0.649	0.001–0.154
India	Sharma *et al.* (2007)	0.0089	0.0218	0.0058	0.002	0.03	0.0367	0.0051
India	Nag and Raikwar (2008)	0.0188	0.0985	0.0101	0.0346	0.162	0.1724	0.0492

[a] ND = not detected.

The contamination of cow milk with dieldrin has also been reported from Japan, although the levels were low, varying from traces to 0.01 mg/kg (Uyeta *et al.*, 1970; Tomizawa, 1977). In another report, the highest dieldrin residues in cow milk were recorded as 0.02 mg/kg (Tanabe, 1972), although no aldrin or endrin was detected in any of the samples. The dieldrin contamination of cow milk has also been reported from Israel, with an average level of 0.01 mg/kg (Veierov *et al.*, 1977). Other contaminants detected at low levels in a few milk samples from Israel include aldrin, heptachlor and heptachlor epoxide. Twelve of 23 milk samples from Bombay contained dieldrin at an average concentration of 76 μg/g on a fat basis, which was 500 times greater than the MRL of 0.15 μg/g (Khandekar *et al.*, 1981). All the 30 cow milk samples collected from different markets in Madrid (Spain) in 1990 and 1991 contained PCBs while heptachlor epoxide was present in the majority of the samples (Hernandez *et al.*, 1994). The results of Kannan *et al.* (1992) on persistent organochlorine residues in food-stuffs from India and their implications for human dietary exposure suggested very low levels of PCBs (1.7–210 ng/g on a fat basis) in bovine milk. The levels of aldrin, dieldrin and heptachlor were also very low (0.06 to 8.1 ng/g on a fat basis).

Out of 192 cow milks collected from the central region of Veracruz State, Mexico, HCB residue was found in 173 samples with a mean concentration of 0.025 mg/kg. However, aldrin, heptachlor and its metabolite heptachlor epoxide were not present above their detection level (Waliszewski *et al.*, 1996). During their three-year monitoring programme, Wong and Lee (1997) collected 252 milk samples from local Hong Kong markets and detected HCB in 55% of samples (mean level of 0.07 mg/kg), dieldrin in 45% of samples (mean level of 0.08 mg/kg) and heptachlor epoxide in 11 samples (mean level of 0.08 mg/kg). Aldrin residue with a mean concentration of 0.035 mg/kg was found in nine milk samples out of 72 collected from supermarkets in Beijing, China, during 2001 (Zhong *et al.*, 2003). The Organic Centre revealed that out of 739 conventional milks tested by the USDA's (United States Department of Agriculture's) Pesticide Data Program 41% contained dieldrin. They also reported on the other OCs, such as DDE which occurred in 96% of samples and endosulfan sulfate which was found in 18% of samples (http://www.grist.org/article/got-chemical-and-pesticide-residues-in-your-milk/).

Incidences of HCB residues above the detection limit of 0.001 mg/kg on a fat basis in cow milk were noticed in 63% of samples in 1998 and in 62% of samples in 2001 during a survey conducted on 150 samples each year from several farms surrounding the city of Veracruz, Mexico. The mean level of HCB was 0.008 and 0.006 mg/kg in 1998 and 2001, respectively (Waliszewski *et al.*, 2003).

Other than OCPs, the persistent organic pollutants (POPS) include poly-brominated diphenyl ethers (PBDEs), polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), polychlorinated biphenyls (PCB), etc. PCBs were introduced in 1929 and were first detected in bovine milk in 1970. They are extremely stable and undergo biomagnifications, i.e., their

bioaccumulation increases at every trophic level. The dioxins and furans preferentially accumulate in the liver of the organism as revealed by the liver adipose tissue ratio of different organisms in different studies. The most known and toxic in this group is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is a known human carcinogen and endocrine disruptor. TCDD is a ligand for the aryl hydrocarbon receptor (AhR) and blocks the binding to the OR radical. The toxicity of other dioxins and chemicals like PCBs that act like dioxins is measured in relation to TCDD in units called tetrachlorodibenzo-*p*-dioxin equivalents (TEQ) by comparing their relative binding to the AhR receptor.

Organophosphate pesticide residue (OPPR)
The highly persistent organochlorinated pesticides, after their banning or restriction in use, have been replaced mostly with organophosphate (OP) compounds which have wide application, mainly as insecticides. Organophosphates are characterised as less persistent and are easily metabolised in the system, although they are highly toxic to mammals. These substances are mainly acetylcholine esterase inhibitors with associated neurological and neuro-muscular effects. They are also carcinogens, teratogens and mutagens.

In spite of being less stable and less persistent than the OCPs, there are some reports alluding to the presence of OP residues in milk. A survey of milk for the presence of pesticide residues in European countries has shown that the contamination was very low in relation to their MRL (IDF, 1991, 1997). However, some of the less polar and fat-soluble OPs (like acephate, diazinon, phorate, methamidophos, chlorfenvinphos, chlorpyriphos, ethion, etc.) were found in foods with high fat content including dairy products (Ivey *et al.*, 1993). Baldi *et al.* (1979) reported chlorfenvinphos (0.002 μg/g) and diazinon (0.001 μg/g) in milk samples from European countries. Abd-Alla *et al.* (1991) showed the presence of lannet (0.038 μg/g) and malathion (0.08 μg/g) in buffalo milk samples collected from Egypt. However, Lino and Noronha da Silveira (1992) could not detect any residue of *cis*-mevinphos and methyl parathion in 25 milk samples during 1992 from Mondego, Portugal but the oxygen analogue of parathion, i.e. paraoxon, was present in 22 samples at concentrations ranging from 1.5 to 8.7 ppb.

Approximately 40% of the 96 samples of Mexican pasteurised milk contained detectable levels of OP residues. Concentrations of the residues in 84% of the positive samples were at levels below the corresponding Mexican limits. Eight samples contained residues exceeding established MRLs and OP pesticides present in these samples were dichlorvos, phorate, chlorpyriphos and chlorfenvinphos. Residues of diazinon, chlorpyriphos and malathion at levels of 0.005–0.586 mg/kg, 0.0256 mg/kg and 0.11 mg/kg, respectively have been reported in milk (El-Kohly *et al.*, 2000; Szerletics *et al.*, 2000; El-Hoshy, 1997). The presence of chlorfenvinphos residues in raw milk has been observed by Kituyi *et al.* (1997). The average values of 13 OP pesticides measured were below established MRLs, ranging from 0.0051 to 0.0203 ppm (Salas *et al.*, 2003). Chlorpyriphos (0.01–1.35 mg/kg) was detected in 20 samples, all exceed-

ing the MRL, out of 324 pasteurised milk samples analysed from Ludhiana (Punjab, India) according to Cheema *et al.* (2005). Residues of different OPs were recorded in 136 samples out of a total of 325 bovine milk samples analysed from the Bundelkhand region of India. The main contaminant was chlorpyriphos which was present in 77 samples in concentrations ranging from 0.007 to 0.064 mg/kg, and the MRL of 0.01 mg/kg was violated in 48 samples. Quinalphos, malathion, monocrotophos and chlorpyriphos-methyl were the other OPs recorded (Nag and Raikwar, 2006).

Among the 135 raw milks collected in four Italian dairy plants, 37 were positive in traces and 10 showed an OP contamination ranging from 5 to 18 μg/kg, which was lower than the MRL fixed by the EU. The higher results were recorded in the samples collected during the autumn–winter period and the main OPs detected were acephate and chlorpyriphos (Pagliuca *et al.*, 2006). Excepting malathion (0.018 mg/kg), which was present in a single sample, there was no contamination with other OP pesticide residues in 40 cow milk samples collected from Gharbia Governorate of Mexico during 2005–06 (Nasr *et al.*, 2007).

Pesticide residues in butter and ghee

Butter has often been found to be contaminated with OCPs in India. All the eight samples of butter from Delhi were excessively contaminated with DDT (Agnihotri *et al.*, 1974). The residues in branded butter varied from 1.1 to 8.0 mg/kg while the unbranded local butter revealed a contamination level of 2.8 to 3.8 mg/kg. Duggan and Duggan (1973) reported that only five out of 1141 samples of the dairy products analysed in the USA contained DDT residues above 2 mg/kg, while 80 and 48 of the 145 butter samples contained more than 2 mg/kg DDT and HCH, respectively, in India (Kalra and Chawla, 1985). Residues of HCH (0.02–11.97 mg/kg) and DDT (traces to 16.04 mg/kg) were found in all the 105 popular brands of butter originating from different parts of India during 1978–81 (Kalra *et al.*, 1983). The DDT level in 33 samples was above the MRL of 1.25 mg/kg, and the mean HCH concentration of 1.89 mg/kg exceeded the maximum level in this commodity reported by 12 countries to the International Dairy Federation (Downey, 1972). The overall results from most European countries also demonstrated that the levels of DDT, HCH, aldrin, dieldrin and heptachlor were negligible (Downey, 1972).

Kaphalia *et al.* (1990) found DDT and HCH residues in all the eight butter samples collected from different parts of India and the maximum levels were 4.9 mg/kg and 1.25 mg/kg, respectively. HCH levels in butter from Bombay, Delhi and Madras in 1990 averaged 2.5, 2.5 and 4.5 μg/g, respectively, and the corresponding values for DDT were 0.92, 2.0 and 1.1 μg/g (Kannan, 1994). Similarly, Shah *et al.* (1992) found all four butter samples from Gujarat, India, contained DDT and HCH residues. The values of ΣHCH and ΣDDT ranged from 0.86 to 3.68 mg/kg and from 1.65 to 5.84 mg/kg with a mean level of 2.04 and 3.55 mg/kg, respectively. While HCH was below the MRL, the MRL for DDT was exceeded in all the samples. All five butter samples from Jabalpur, India, were found to contain DDT and HCH residues. The concentrations were

above the MRL for DDT in three samples and for HCH residues in all samples (Mitra *et al.*, 1999).

A study (Kalantzi *et al.*, 2001) on the global distribution of PCBs and OCPs in butter revealed that concentrations of pp[l] DDT, pp[l] DDE and HCH isomers all varied over many orders of magnitude, with the highest levels being found in the areas of current use, i.e. India and South/Central America for DDT, and India, China and Spain for HCH. The organochlorine pesticide residues determined in 200 butter samples during 2001 in Mexico were low and within the tolerance limits. HCB was found in 99% of samples, having a mean level of 0.008 mg/kg. Among HCH isomers β-HCH was the main contaminant at 0.065 mg/kg, while among the DDTs, pp DDE was present in 100% of samples at a mean concentration of 0.043 mg/kg followed by pp[l] DDT and op[l] DDT. The comparison of monitoring studies carried out in 1994 and 2001 indicates a diminution of OCP residue levels in butter (Waliszewski *et al.*, 2003). Analysis of 40 butter samples collected from the Ludhiana district of Punjab showed the presence of DDT and HCH in 28 and eight samples, respectively. DDT was found in the form of pp[l] DDE and pp[l] DDD while γ-HCH was mainly present among the HCH isomers (Battu *et al.*, 2004). None of the samples revealed the presence of any commonly used organophosphorus or synthetic pyrethroids at their detection limit of 0.01 mg/kg.

Out of 11 local butter samples collected from markets in Jorhat in Assam, three samples were contaminated with pp[l] DDE (0.051–0.069 mg/kg) and γ-HCH (0.049–0.058 mg/kg), and one each with pp[l] DDT (0.055 mg/kg), α-HCH (0.045 mg/kg), α-endosulfan (0.078 mg/kg) and endosulfan sulfate (0.075 mg/kg) (Deka *et al.*, 2004). Nearly 94% of the samples of all local commercial brands of butter sold in a supermarket in Konya, Turkey, were found to be contaminated with one or more OCPs, mainly DDT complex and HCH isomers. Aldrin, dieldrin, endosulfan, endrin and heptachlor were also detected in a few samples (Nizamlioglu *et al.*, 2005). Not only OC pesticides but also PCB residues have been found in butter. Goni *et al.* (1994) recorded about 20–60 ng/g PCB residues in butter samples in Spain.

Ghee is a clarified semi-fluid butter used raw as well as in Indian cooking. It has often been found to be excessively contaminated with OCPs. Kalra *et al.* (1983) found all the five ghee samples from Ludhiana, India, were contaminated with DDT above the MRL, while the HCH level varied widely from 0.30 to 6.65 mg/kg. All the 42 samples of ghee from Lucknow, India were contaminated with HCH (1.29–1.42 mg/kg) and DDT (4.47–9.86 mg/kg) (Lata *et al.*, 1984). In a monitoring study conducted at Anand, Gujarat, by Shah *et al.* (1992) it was observed that 29 popular brands and 23 local brands of ghee were contaminated with DDT and HCH isomers. The levels of total HCH and total DDT ranged from 0.02 to 4.96 mg/kg and from 0.02 to 7.62 mg/kg, respectively, with a mean of 1.84 and 2.38 mg/kg, respectively (Shah *et al.*, 1992). Again, from different locations in Uttar Pradesh, India, DDT and HCH at concentrations up to 9.8 and 3.8 mg/kg, respectively, were reported in ghee samples (Kaphalia *et al.*, 1990).

Baby milk powder

The presence of residues of HCH and DDT in infant food is of particular concern, since newborn children often depend on them for a substantial period of time. Different brands of infant formula from Gujarat, Maharashtra and Punjab in India were found to be contaminated with DDT above the MRL (Dhaliwal and Kalra, 1978). Infant food collected from Ludhiana was found to be 100% contaminated with DDT, 90% of which were above the tolerance level (Shastry, 1983). The presence of DDT and HCH residues in different brands of baby milk powder was also reported by Kumar *et al.* (1992) and Kathpal *et al.* (1992). Gupta *et al.* (1997), however, did not find any residues of either DDT, α-HCH or δ-HCH in six milk powders collected from Jaipur. Only one milk powder was found to contain β- and γ-HCH. In the analysis of baby milk powder collected in India from Solan (Himachal Pradesh), Hyderabad (Andhra Pradesh), Trivandrum (Kerala), Kolkata (West Bengal), and Bangalore (Karnataka), it was found that HCH was the major contaminant (Agnihotri, 1999). Among different isomers of HCH, the β isomer was found to be the most prevalent followed by α, γ and δ isomers. In another multi-centre collaborative Indian study conducted by Kalra *et al.* (2001) in which 186 samples of 20 different infant formulas collected from Mumbai, Pune, Mysore, Lucknow and Ludhiana were analysed to determine the level of DDT and HCH residues, it was observed that 70–94% were contaminated with DDT and HCH. The average concentration was 0.30 and 0.49 mg/kg on a fat basis for DDT and HCH, respectively.

5.3   Heavy metal pollution in milk

Bioaccumulation of toxic heavy metals, e.g. lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), etc., in milk has evoked great concern in recent years, just like the presence of any other toxic matter in milk. That is why health authorities and the public have given special attention to the risks posed by contamination of food with toxic heavy metals. Heavy metal contaminants enter animal systems through pollution of air, water, soil and feeds. Once in the animal, these metals can persist for several weeks even after discontinuation of the exposure. The accumulation of heavy metals in dairy animals adversely affects health and milk production (Dey *et al.*, 1996; Dogra *et al.*, 1996). It has been found through experiment that the cardiovascular and immune systems are affected before the appearance of clinical symptoms caused by heavy metal poisoning (Dey *et al.*, 1993; Haneef *et al.*, 1995). The toxic metals mercury, lead and cadmium have reproductive and endocrine system disrupting effects.

Elevated levels of lead could be detected in animal tissues up to 12–19 months after exposure has ceased (Hatch, 1988). In his study on steady-state bovine milk bio-transfer factors for disposition of toxic metals in the agricultural food chain, Stevens (1991) reported that Pb and As transfer to milk to the greatest extent, followed by Cr, Hg and Cd. Goski and Nikodemska (1991)

concluded that levels of Pb and Cd in cow milk from industrialised regions of Katowice, Poland, from 1988 to 1999 were much higher than in milks from agricultural regions. The mean concentration of toxic elements in bovine milk in the Polish district of Zgorzelec-Bogatynia was low, but in individual samples Pb concentrations (0.252 mg/kg) greatly exceeded those from other regions (Zmudzki *et al.*, 1992). Normally, very low concentrations of these metals are secreted into milk because the carryover rate of metals from forage to milk is approximately 1:500 (Bluthgen *et al.*, 1997). The body has no homeostatic mechanism for regulating these metals in tissues. Some of the heavy metals, e.g. As, Cd, Hg and Pb, are cumulative poisons and are toxic even at a low concentration. A higher Pb burden in blood and milk from animals reared in urban localities and around polluting industrial units in various parts of India (Swarup *et al.*, 2004) and elsewhere in the world (Baars *et al.*, 1990) has been documented. Milk Pb concentration is exponentially related to blood Pb (Okada *et al.*, 1997; Swarup *et al.*, 2004). It is expected that animals exposed to industrial Pb will excrete higher amount of Pb in milk.

Because of its toxicity and passage into milk, Codex International has established specifications for Pb in milk and milk products (0.02 ppm) and in butter (0.05 ppm). Higher concentrations of Pb in milk and blood were reported in some Indian cities such as Delhi (Dey *et al.*, 1996), Varanasi (Bhatia and Chowdhury, 1996) and Kanpur (Swarup *et al.*, 1997). Lead levels in milk were found to be normal in different areas of Gorakhpur, India (Singh and Kumar, 1996). However, high concentrations of Cd due to a polluted environment were found in milk from Kanpur, India (Swarup *et al.*, 1997). The organic forms of Cd are unstable but highly toxic. Root uptake by forage plants, especially at lower pH, is the main route of Cd uptake into the food chain.

Decun *et al.* (1995) concluded that the Pb content of milk and tissues in western Romania might constitute some danger but only in cows from certain heavily industrialised regions. In three regions of Lower Silesia, Poland, known to be contaminated with heavy metals due to copper mining and the chemical industry, analysis of milk from 21–37 cows revealed that 19–68.6% of samples were contaminated with Hg and 17.1–27.0% of samples with Se at levels exceeding the permitted concentration, and there was also a significant correlation between Hg and Se concentrations (Kolacz *et al.*, 1996). The concentration of Cd and Pb in 103 bulk milk samples collected at five dairies in the vicinity of the Bogdaenka coal mine, Poland, in 1998 was 6.62 and 0.96 μg/litre, respectively, which was within the limits suitable for human consumption, though significantly higher than in milk samples from agricultural areas (Litwinczuk *et al.*, 1999). A study on transfer of heavy metals along the soil–ration–milk chain in farms in Moscow, Russia, during 1995–98 revealed that the concentrations of elements such as Zn, Cu, Cd and Co were lower than the maximum tolerated concentration (MTC), but Pb exceeded the MTC in milk from some farms. The highest level of migration of the elements from soil to milk was seen in podzolic, boggy, stratified soil, and the minimum was found from floodplain soils (Sirotkin *et al.*, 2000). Analysis of 75 samples of milk from three different

regions (an industrial, a rural and a heavy traffic-intensive region) around Bursa, Turkey, revealed average amounts of 0.032, 0.049 and 0.018 mg/kg of Pb, 0.05, 0.009 and 0.0002 mg/kg of As, 4.49, 5.01 and 3.77 mg/kg of Zn, 0.58, 0.96 and 0.39 mg/kg of Cu, and 1.78, 4.27 and 1.01 mg/kg of Fe, respectively. However, mercury was not found in any sample, and the highest heavy metal content was found in samples from the industrial region, followed by the traffic-intensive region and the rural region (Simsek *et al.*, 2000).

According to a survey conducted in two villages (declared to be arsenic-contaminated) in two districts of West Bengal, India, feed roughages given to animals contained highly toxic levels of As. According to a rough estimate, lactating animals were consuming 44 mg/d of As from all feed sources, including water, which was far from tolerable levels and, as a result, the average excretion of As in milk was found to be 77 ppb (0.077 mg/kg) (Singh *et al.*, 2002). In another report (Rana *et al.*, 2008) from a high arsenic-contaminated area in the Nadia district of West Bengal, the concentration of As in milk was found to be 0.156 mg/kg. Ozdemir *et al.* (2009) found that the Pb content (3.24–80.69 μg/kg) in milk of cows grazing at the roadside pastures in Sakarya, Turkey, polluted due to vehicle emissions, were higher than the regulatory limits, though Zn levels (1262–6566 μg/kg) were safe.

5.4   Radionuclides

Radioactive elements may be present in soil naturally or by accumulation from industrial wastes. Radionuclides are radioactive isotopes of certain elements. Such isotopes are always present in milk but in minute quantities. Their presence in milk is attributed mainly due to passage from grasses and fodder to milk. Plants get contaminated either by uptake from contaminated soil or through deposition on a leaf surface. Radionuclides enter the milk and milk products through naturally occurring radioisotopes (Yousef and Marth, 1985). The potentially hazardous radionuclides in milk are ^{90}Sr, ^{89}Sr, ^{131}I, ^{133}I, ^{134}Cs, ^{140}Ba and ^{40}K. Among the naturally occurring radionuclides ^{40}K is the most important and is present at a higher level. It is more hazardous due to the higher energy of radiation as compared to ^{137}Cs. Natural radioactivity due to ^{40}K present in milk powders is in the range of 600–800 Bq/kg, while in liquid milk it is in the range of 30–45 Bq/litre for Indian milk and 50–80 Bq/litre for foreign milk. It has also been reported that cow milk contains 44 Bq/litre and buffalo milk contains 38 Bq/litre. Since potassium salts are very soluble, ^{40}K is retained in the liquid phase in the preparation of butter, which therefore contains only 1.3 Bq/litre (Merai and Boghra, 2004).

Among the artificial radioactivity arising from activities using nuclear materials, the important elements from the point of view of dose via food consumption are ^{90}Sr, ^{134}Cs, ^{137}Cs and ^{131}I. Total radiocesium contamination in milk, cheese and buttermilk samples collected in regions of Russia, Belorussia and the Ukraine, heavily contaminated by the Chernobyl accident, ranged from 1

to 170 Bq/kg, and the level of ^{90}Sr was 1.8–30 Bq/kg. In milk, radio-strontium and radio-cesium occur only in the aqueous phase, while minute quantities of ^{131}I are attached to milk fat. So, a far smaller amount of the radionuclides present in milk will be found in cream and butter. Since the physical half-life of ^{90}Sr is very high, exposure to its harmful radiation is prolonged in comparison with others. It is distributed in milk in much the same way as calcium. Iodine is found in milk serum, a small portion being strongly bound to proteins. Cesium chemically behaves like Na^+ and K^+. The ratio of ^{90}Sr to ^{137}Cs in European milk is 1.8, while it is 0.5–1.58 in Indian milk. In India, ^{137}Cs levels have been reported to be 0.02 Bq/litre in milk and 0.6 Bq/kg in milk powder. Most dairy products generally contain ^{137}Cs to levels of 40 Bq/kg while ^{131}I is present to concentrations of 20 Bq/litre (Mathur *et al.*, 1999).

In respect of standards of radioactivity set by the Atomic Energy Regulatory Board (AERB), radiation exposure from milk and milk products alone is quite small as compared to natural and other sources of radiation exposure that is received during daily activity.

5.5   Veterinary drug residues

Modern veterinary drugs are used in livestock production to reduce mortality and morbidity from infections, to increase growth rates, to relieve stress or to tranquillise, to improve feed conversion, to increase milk secretion and to increase productivity in general. The veterinary drugs and active compounds associated with milk are chemotherapeutics (antibiotics and sulfonamides), endoparasiticides (fasciolicides and antihelminthics), ectoparasiticides, hormones, and teat and skin disinfectants (iodophores, chlorphenidine, quats, linear alkyl benzyl sulfate). Antimicrobial residues in milk should be taken into account for health aspects such as possible impact on the emergence of antimicrobial resistance against antimicrobials administered in human therapy, disorders of the intestinal flora and possible occurrence of allergic symptoms. Antibiotic residues in milk may lead to severe allergic reactions in sensitive consumers. In the dairy industry, antimicrobial residues cause economic losses. Apart from the concern to public health, the presence of residues has created other problems in the dairy industry, including inadequate curdling of milk during manufacture of curds and yoghurt, inadequate ripening of cheese, etc. In fermented milk products, the effect of antimicrobials slows or inhibits acid formation and results in inadequate aroma formation. Even low levels of residue below the MRL can cause defects in cheese, including off-flavours, uneven texture, uneven eye development and a tendency for butyric acid fermentation. As a preventive measure in most countries, milk is withheld for fixed times and/ or milk containing antimicrobials is discarded so that drug residues are not present or drop below the permissible level.

There are several different groups of antibiotics available to treat infected dairy cattle. The most common groups include the β-lactams (e.g. penicillin,

cephapirin), sulphonamides (e.g., sulfamethazine), aminoglycosides (e.g. streptomycin, gentamycin), tetracycline (oxytetracycline), macrolides (e.g. erythromycin), quinolones (e.g. fluroquinolone), etc. These antibiotics may be used singly or, at times, in combination when treating dairy cattle for certain diseases like mastitis and others. Dairy cattle that have been treated with antibiotics produce milk containing antibiotic residues for a period of time after treatment. Milk from treated cows is therefore required to be excluded from the milk supply for a specific time period to ensure that antibiotic residues no longer persist in their milk. Antibiotic residues enter the milk supply when treated cows are returned to the milking herd early or when a cow retains antibiotic residues in her system for a long time.

The excretion of a residue in the mammary gland depends on the degree of its ionisation, its solubility in fat and water and on the difference between the pH of plasma and milk. Lipid-soluble antibiotics which ionise as bases move readily from blood to milk. Organic bases ionise at the pH of milk and cannot diffuse back towards the blood. The level of drug residues in milk is also affected by their route of administration. In a study to determine whether extra-label use, the route of administration or the type of drug is responsible for prolonged shedding of residues in milk, it was found that extra-label use of antibiotics was significantly associated with increased risk of antibiotic residues in milk beyond the label withholding time. No significant differences in risk were observed among the various antibiotic products used in the study, but the route of administration had an effect, as the proportion of antibiotic residue positive samples was significantly higher when given subcutaneously rather than by other routes, such as intramuscular, intravenous, intramammary, etc. (McEwen et al., 1992).

There are only a few published studies available on the occurrence and level of antibiotic residues in milk samples. About 1.5% of 337 samples obtained from stores in Europe were found to contain detectable antibiotics when analysed for the antibiotic residues by the microbial receptor assay and by four different microbial inhibitor assays. Penicillin, chloramphenicol and gentamycin were detected in seven, one and two samples, respectively (Suhren et al., 1990). Sudarshan and Bhat (1995) detected oxytetracycline in 9% of market milk samples collected from Hyderabad and Secunderabad in India. Two of the 15 pooled milk samples collected from five public milk booths from Guwahati were reported to contain antimicrobial substances (Dutta et al., 2001). The screening of a total of 2686 raw ewe milk samples from different ovine dairy farms from the Castilla la Mancha region of Spain showed 1.7% positive and 2.1% doubtful samples, which decreased after heating to 1.3% and 0.4%, respectively. Positive and doubtful samples were identified by penicillinase and p-aminobenzoic acid solution (Yamaki et al., 2004). About 37% of 137 unprocessed milks collected from Hyderabad, Latifabad and Qasimabad, Pakistan, were positive for β-lactam antibiotic residues. The levels of residues were 0.4–400 μg/litre for Penicillin G, 1.0–190 μg/litre for amoxicillin, 0.5–141 μg/litre for ampicillin, and 2.1–122 μg/litre for unknown antibiotics. The mean concentrations of penicillin, amoxycillin

and ampicillin were significantly higher than the MRL proposed by the EU (4 μg/litre) and FDA (5 and 10 μg/litre) (Khaskheli *et al.*, 2008). A survey of 205 milks collected from Karnal, India, during 2006 depicted an incidence of antibiotic residues up to 20.45% at MRL Codex limits, with individual presence of β-lactam 2.43%, sulpha drug 1.95%, tetracycline 0.48%, amino-N 0.00, amino-ST 7.31%, macrolide 1.46% and multiple drugs 6.82% (Kumar, 2009).

5.6   Mycotoxins

The word mycotoxin is derived from myco, meaning mould, and toxin, a poison. Mycotoxins or fungal toxins are low molecular weight secondary metabolites (i.e., metabolites not essential to the normal growth and reproduction of the fungus) formed by a consecutive series of enzyme-catalysed reactions from a few biochemically simple intermediates of primary metabolism in a wide range of fungi, particularly moulds. There are about 400 secondary metabolites of moulds but only a few of them are toxins causing pathological, physiological or biochemical alterations in other species, including humans and animals. Such poisoning is referred to as mycotoxicosis.

There are over 100 known species of moulds that produce mycotoxins, but most of the mycotoxins of importance are produced mainly by the species of three genera, viz. *Aspergillus*, *Penicillium* and *Fusarium*. The most studied mycotoxins are aflatoxins (AFB1, AFB2, AFG1, AFG2, AFM1 and AFM2), ochratoxins, fumonisin, zearalenone and trichothecenes, particularly deoxy-nivalenon (DON), diacetoxyscirpenole (DAS), nivalenone (NIV), T-2 and HT-2 toxins. Mycotoxin contamination of animal fodder occurs in the field owing to infestation by particular pathogenic fungi or with symbiotic endophytes and also during processing and storage of harvested products and feed in appropriate environmental conditions. In general, mould growth and mycotoxin production require a moisture content of greater than 14%, a temperature of at least 25ºC and some degree of aeration. When these conditions are met, mould infestation and mycotoxin production in target crops are likely to occur. During the process of host colonisation, fungi may produce toxins that are harmful for humans and animals. This occurs mainly under specific conditions of plant stress, such as those produced by adverse weather conditions. For instance, drought has been shown to prompt aflatoxin production in groundnuts. Differences in mycotoxin production are also related to plant genotype. Clinical manifestation of myco-toxicoses in animals depends on exposure, concentration and type of mycotoxin. Differences in tolerances to mycotoxins exist among poultry, pigs and cows.

The most important mycotoxin is aflatoxin (bisfurano coumarins) produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. The aflatoxins are potent carcinogenic, mutagenic, teratogenic and immunosuppressive agents and cause liver damage, decreased milk yield and egg production, and suppression of immunity in animals. Aflatoxin-producing strains of *Aspergillus* are widely distributed in soil and air. Under favourable environmental conditions, colon-

isation and mould growth can easily occur in substrates like feed or seed. The nature and concentration of aflatoxin will vary greatly according to prevailing weather conditions, substrate and the type of mould. *A. flavus* produces primarily aflatoxin B_1 and B_2 on corn, while *A. parasiticus* on corn produces all four major aflatoxins B_1, B_2, G_1 and G_2. Three major feedstuffs, viz. corn, cottonseed and peanuts, have high potential for invasion by *Aspergillus* spp. during growth, harvest, transportation or storage. In the case of soybean and small grains, colonisation generally occurs during storage. Poor storage conditions, high moisture and temperature, lack of aeration, kernel damage and spore dissemination caused by storage insects, etc., aid in colonisation and promote aflatoxin production. Chronic poisoning or aflatoxicosis can result when low levels of toxins are ingested over a prolonged period. The toxic effects are not nearly as specific or clinically evident as in acute intoxication. In general, affected livestock exhibit decreased growth rate, lower productivity and immunosuppression. Among the biological effects, aflatoxins have an effect on protein synthesis as they interact with a number of different cell components involved in the process. Aflatoxins have a high affinity for nucleic acids and polynucleotides (RNA and DNA), particularly at nucleophilic sites on the guanine base. Formation of these aflatoxin–nucleic acid covalently bonded adducts can lead to a number of different biological effects, including cancer. Aflatoxin is carcinogenic in several species including rats, ducks, mice, trout and primates. AFB1 is considered the most potent carcinogen known, with AFG1, AFB2 and AFG2 in order of decreasing potency.

Aflatoxins are the major mycotoxins known to enter milk and many milk-based foods. The naturally occurring aflatoxin B_1 in feed is bio-transformed by mammals into various metabolites including aflatoxin M_1 (a hydroxylated derivative of AFB1, also known as milk toxin) excreted in milk, which is hepatotoxic and hepatocarcinogenic. The acute toxicity of AFM1 is similar to or slightly less than that of AFB1 but its carcinogenic potential is probably one or even two orders of magnitude lower than that of AFB1 (Henry *et al.*, 2001). AFB1 has been categorised by the IARC (International Agency for Research on Cancer) as a class I human carcinogen and AFM1 as a class IIB human carcinogen. The presence of AFM1 in cattle and buffalo milk and milk products was reported from various parts of India (Paul *et al.*, 1976; Patel *et al.*, 1981; Shemakayani and Patel, 1989; Tiwari and Chauhan, 1991; Rajan *et al.*, 1995, Choudhary *et al.*, 1997; Dhand *et al.*, 1998; Vaidya *et al.*, 1998). The extent of contamination varied from very few to 94.17%. The average level of AFM1 in buffalo milk was lower than that in cow milk, probably due to the lower conversion of AFB1 to AFM1 through hydroxylation in buffaloes. AFM1 was detected in 142 of 1465 samples (9.6%), 35 of 1533 samples (2.3%), 104 of 1697 samples (6.1%) and 343 of 1750 samples (19.6%) collected from 50 premises in each of four different districts of Bavaria (Germany) during May 1987 and April 1988 (Bachner *et al.*, 1988). Saad *et al.* (1989) found that six of 20 samples of camel milk collected from several sources in Abu Dhabi contained AFM1 at levels ranging from 0.25 to 0.8 ng/ml. AFM1 was detected

in 30% of the milk samples at levels ranging from 4 to 23 ng/l and in 13% of the cheeses at levels from 21 to 101 ng/kg when 40 sheep milk and 30 cheese samples in western Sicily were analysed (Finoli and Vecchio, 2003).

Besides AFM1, other aflatoxin metabolites, viz. M2, M4 and B1, are also excreted in milk (Cheeke and Shull, 1985). The concentration of AFM1 in milk increases proportionally with the amount of AFB1 in the diet. About 1–6% of the ingested dose of AFB1 appears in milk as AFM1. The carryover rate, however, can vary in individual animals, with the concentration of AFB1 in feed, with duration of feeding of contaminated ration, from day to day and from one milking to another milking, as it is influenced by feeding regime, health status, individual capacity for bio-transformation, etc. When ingestion is continuous, the concentration in milk will increase until equilibrium with intake is established. Among the defined mycotoxins carryover has been analysed and quantified only for a small group of fewer than 10 compounds. AFM1 has the highest carryover into the milk of lactating cows with 0.1% of the toxin ingested with the feed in the form of AFB1 per litre of daily milk yield. Of the other tested mycotoxins, only one, fusarium toxin of the type trichothecene A, the T-2 toxin, displays a carryover rate of 2 mg/litre after an unrealistic high dosing in an unnatural feeding regime (Bluthgen *et al.*, 2004). Fermentation of the feed due to storage in damp conditions may induce formation of mycotoxins and their transmission into milk. Samples of cow milk collected from some areas of Bangladesh where fermented rice straw was used for feeding confirmed that dampness leads to the transmission of AFM1 from fermented straw to the cow milk, albeit at low levels (0.001–0.006 μg/kg) (Bhuiyan *et al.*, 2003). AFM1 in the range of 2.04–4 μg/litre was noticed in milk and ice-creams in Abeokuta and Odeda local governments of Ogun state of Nigeria (Atanda *et al.*, 2007).

5.7   Nitrates and nitrites

Nitrates are a naturally occurring form of nitrogen and an integral part of the nitrogen cycle. Manures, nitrogenous fertilisers, decaying plants and animals and other organic residues generate nitrates, which are also produced in the human and animal body. Due to increased use of fertilisers and manures in intensive crop production systems, the level of nitrates is increasing in cereals, other field crops and water. Forage crops may also be a source of high levels of nitrates. Sudan grass, oats, rape, wheat, barley and maize accumulate nitrate. Nitrate *per se* is relatively non-toxic, but approximately 5% of all ingested nitrate is converted in saliva and the GI tract to the toxic nitrite by bacterial enzymes. Nitrite and N-nitroso compounds formed by binding nitrite to other substances like amines, amides, etc., are toxic and can lead to severe pathologies in humans. N-nitroso compounds have also been reported to be carcinogenic in more than 40 animal species, including mammals, birds, reptiles and fish. The best known effect of nitrite is its ability to react with haemoglobin (Oxyhb) to form methaemoglobin (metHb) and nitrate. MetHb cannot bind oxygen and as a

result oxygen delivery to tissues is impaired. Once the proportion of metHb reaches 10% of normal Hb levels, clinical symptoms like cyanosis and asphyxia occur. Nitrate reduction in the rumen competes with other metabolic reactions such as methanogenesis and results in the formation of other end products, leading to abortion, infertility and goitre. Only good agricultural practices can negate this problem.

Although milk and milk products are, either secretory or post-secretory, contaminated with nitrates, nitrites or nitrosamines, the level does not pose any health risk. The nitrate level in milk is naturally relatively constant in the range of 1–12 mg/kg raw milk, while nitrite is practically absent. Traces of volatile nitrosamines can be detected in milk. The nitrate and nitrite residues in milk were studied after application of KNO_3 to dairy cows at two-week intervals in single peroral doses of 75, 37.5, 18.75 and 9.5 g two hours before evening milking. A marked increase in nitrate content appeared in dependence on applied KNO_3 up to 38 hours, and in samples taken at 50 hours the level was at par with the control. The average value of residual nitrate in milk at two hours after administration of 150 g of KNO_3 was 34.60 mg $NO_3{}^-$/litre. The nitrate concentration, however, did not exceed 0.05 mg/litre in any single sample (Baranova *et al.*, 1993). Priyo and Contin-Esnault (1996) surveyed nitrate contents in Indonesian milk; nitrate was detected in the range of 1–2.6 mg/kg in fresh milk and 1.1–18 mg/kg in dry milk. All of 185 Turkish cheese samples made from cow and sheep milk were found to contain nitrate, in the concentration range of 0.47–23.68 mg/kg, while nitrite was detected in 88.11% of samples, having a mean value between 0.88 and 1.64 mg/kg (Topcu *et al.*, 2006).

5.8   Detergents and disinfectants

Detergents and disinfectants are used in milk production for cleaning and sanitation at various stages. Cleaning of udders of the cows is done with disinfectants. Detergents are necessary to clean milking and ancillary equipment effectively before disinfection. Detergents contain inorganic alkalis (e.g. sodium carbonate and silicates and trisodium phosphate), chlorine and chlorine compounds, iodine and iodine compounds, alcohols, phenolic compounds, nitrogen compounds, surface-active agents (or wetting agents), sequestering (water-softening) agents (e.g. polyphosphates) and acids for de-scaling. Many proprietary, purpose-made detergents are usually available, but otherwise an inexpensive mixture can be made to give a concentration in solution of 0.25% sodium carbonate (washing soda) and 0.05% polyphosphate. The detergents and disinfectants can contaminate milk and its products if proper care is not taken, such as adequate rinsing and drainage of the installations. Impairment of organoleptic quality in milk occurs due to the presence of residues of detergent.

In the next chapter I will discuss the routes through which the various contaminants enter milk and milk products, analytical techniques, regulatory aspects, and techniques to manage the contaminants to minimise their effects.

5.9   References

ABD-ALLA E A M, SAYEED A F and AHMED N S (1991), 'Incidence of some organophosphate pesticide residues in buffalo milk in Giza Governorate', *Egypt J Dairy Sci*, 19, 243–248.

ADIROUBANE D and LETCHOUMANAE S (1996), 'HCH and DDT residues in bovine milk samples from Karaikal region of U.T. of Pondicherry', *Pestic Res J*, 8, 115–118.

AGNIHOTRI N P (1999), *Pesticide Safety Evaluation and Monitoring*, All India Coordinated Research Project on Pesticide Residues, Indian Agricultural Research Institute, New Delhi, pp. 104–107.

AGNIHOTRI N P, DEWAN R S, JAIN H K and PANDEY S Y (1974), 'Residues of insecticides in food commodities from Delhi. II, High fat content food materials', *Indian J Ent*, 36, 203–208.

ALWAI M A and AL-HAWUDI J S (2005), 'Organochlorine pesticides in sheep milk and fodder in Jordan', *J Agric Environ*, 3, 226–228.

ATANDA O, OGUNTUBO A, ADEJUMO O, IKEORAH J and AKPAN L (2007), 'Aflatoxin M_1 contamination of milk and ice cream in Abeokuta and Odeda local governments of Ogun State, Nigeria', *Chemosphere*, 68(8), 1455–1458.

BAARS A J, BEEK HUVAN VISSER I T R, VAN DELFT W, FENNEMA G, LIEBEN G W, LAUTENBERG K, NIEUWENHUIJS J H M, COULANDER P, PLUIMERS F H, VAN DE HAAR G, JRNA T, TUINISTRA L G M T, ZANDSTRA P and BRUINS B (1990), 'Lead poisoning among cattle in North Netherlands between October 1989 and January 1990', *Tijdschrift voor Diergeneeskunde*, 115, 882–90.

BACHNER U, MARTLBAUER E and TERPLAN G (1988), 'Detecting aflatoxin M1 in milk from selected parts of Bavaria by using an ELISA', *29. Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene Dreilandertagung*, 13–16 September 1988, Garmisch-Partenkirchen, Germany, pp. 291–294.

BALDI M, BOVOLENTA A and ZANONI L (1979), 'Food contamination with chlorinated and phosphoric pesticides: two years' research (1977–1978) on foods produced in the province of Ferrara (Italy)', *Industrie Alimentari*, 18, 806–810.

BARANOVA M, MAL'A P and BURDOVA O (1993), 'Transport of nitrates and nitrites into the milk of dairy cows through the digestive system', *Vet Med (Praha)*, 38(10), 581–588.

BATTU R S, SINGH B and KALRA R L (1996), 'Seasonal variation in residues of DDT and HCH in dairy milk in Punjab, India', *Pestic Res J*, 8, 32–37.

BATTU R S, SINGH B and KANG B K (2004), 'Contamination of liquid milk and butter with pesticide residues in Ludhiana', *Ecotoxicol Environ Saf*, 59, 324–331.

BHATIA I and CHOWDHURY G N (1996), 'Lead poisoning of milk – the basic need for foundation of human civilization', *J Anim Health*, 40, 24–26.

BHUIYAN A, AKBAR M A and HOSSAIN M E (2003), 'Nutritive value of damp rice straw and its feeding effect on aflatoxin transmission into cow milk', *Pakistan J Nutr*, 2, 153–158.

BLUTHGEN A, BURT R and HEESCHEN W H (1997), 'Heavy metals and other trace elements', in: *Monograph on Residues and Contaminants in Milk and Milk Products*, International Dairy Federation, Brussels, pp. 65–73.

BLUTHGEN A, HAMMER P and TEUFEL P (2004), 'Mycotoxin in milk production. Occurrence, relevance and possible minimization in the product chain feeds–milk', *Kieler Milchwirtschaftliche Forschungsberichte*, 56, 219–263.

BROWN M A, RUZO L O and CASIDA J E (1986), 'Photochemical conversion of a dicofol impurity, α-chloro-DDT to DDE', *Bull Environ Contam Toxicol*, 37, 791–796.

CHAUHAN R, SINGH Z and DAHIYA B (1982), 'Organochlorine insecticides as food contaminants', *First International Conference on Food Science and Technology*, Bangalore, India, 23–26 May 1982, Abstract No. 9, Section 11, p. 5.

CHEEKE P R and SHULL L R (1985), *Natural Toxicants in Feeds and Poisonous Plants*, AVI Publishing, Westport, CT.

CHEEMA H K, KANG B K and SINGH B (2005), 'Residues of chlorpyriphos in bovine milk in Punjab, India', *Pestic Res J*, 17, 87–89.

CHOUDHARY P L, SHARMA R S, BORKHATRIYA V N, MURTHI T N and WADODKAR U R (1997), 'Survey on the levels of aflatoxin M1 in raw and market milk in and around Anand town', *Ind J Dairy Sci*, 50, 156–158.

COLBORN T, VON SAAL F S and SOTO A M (1993), 'Developmental effects of endocrine-disrupting chemicals in wildlife and humans' *Environmental Health Perspectives*, 101, 378–384.

DECUN M, JULA S and BIRIESCU I (1995), 'Concentration of heavy metals in milk and meat in western Romania', *Revista Romana de Medicina Veterinara*, 5, 271–277.

DEKA S C, BARMAN N and BARUAH A A L H (2004), 'Monitoring of pesticide residues in feed, fodder and butter in Assam', *Pestic Res J*, 16, 86–89.

DEY S, SWARUP D and SINGH G (1993), 'Effect of experimental lead toxicity in cardio-vascular function in calves', *Vet Human Toxicol*, 35, 501–503.

DEY S, DWIVEDI S K and SWARUP D (1996), 'Lead concentration in blood, milk and feed of lactating buffaloes after acute poisoning', *Vet Record*, 138, 336.

DHALIWAL G S and KALRA R L (1977), 'DDT residues in milk samples from Ludhiana and surrounding area', *Ind J Ecol*, 4, 13–22.

DHALIWAL G S and KALRA R L (1978), 'DDT residues in butter and infant formula in India, 1977', *Pestic Monit J*, 12, 91–93.

DHAND N K, JOSHI D V and JAND S K (1998), 'Aflatoxin residues in milk and milk products', *Ind J Dairy Sci*, 51, 129–131.

DOGRA R K S, MURTHY R C, SRIVASTAVA A K, GAUR J S, SHUKLA L J and VARMANI B M L (1996), 'Cattle mortality in Thane district, India: A study of cause/effect relationship', *Arch Environ Contam Toxicol*, 30, 292–297.

DOWNEY W L (1972), 'Pesticide residues in milk and milk products', *Int Dairy Fed Bull*, Part II, p. 51.

DUGGAN R E and DUGGAN M B (1973), 'Pesticide residues in food', in: Edwards E A (ed.), *Environmental Pollution by Pesticides*, Plenum Press, London, pp. 334–364.

DUTTA G N, DUTTA R, JYOTI B and MILLI D C (2001), 'Antibiotic residues in milk after treatment of mastitis', *Ind J Dairy Sci*, 54, 322–325.

EL-HOSHY S M (1997), 'Insecticide residues in milk and influence of heat treatments and bacterial fermentation as safeguard against these pollutants', *J Assiut Med Vet*, 37, 141–155.

EL-KOHLY A F, AFIFI A M, RAGAB A A and EL-BAROTY G S (2000), 'Contamination of buffalo milk with residues of diazinon insecticide after spraying animals', *Vet Med J Giza*, 48, 7–11.

FAO/WHO (1979) Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Codex Committee on Pesticide Residues, Maximum Residue Limits.

FINOLI C and VECCHIO A (2003), 'Occurrence of aflatoxins in feedstuff, sheep milk and dairy products in Western Sicily', *Italian J Anim Sci*, 2, 191–196.

FYTIANOS K, VASILIKIOTIS G, WEIL L, KAVLENDIS E and LASKARIDIS N (1985), 'Preliminary study of organochlorine compounds in milk products, human milk and vegetables', *Bull Environ Contam Toxicol*, 34, 504–508.

GONI F, SERRANO E, IBARLUZEA J M, DORRONSORO M and GALDEANO L G (1994), 'Poly-chlorinated biphenyl residues in various fatty foods consumed in Guipuzcoa, the Basque Country (Spain)', *Food Addit Contam*, 11, 387–395.

GOSKI J and NIKODEMSKA E (1991), 'Contents of lead, cadmium, zinc and copper in cow milk from selected regions of the province Katowice', *Przeglad-Mleczarski*, 1, 18–19.

GUPTA A, PARIHAR N S and SINGH V (1997), 'HCH and DDT residues in bovine milk and milk powder', *Pestic Res J*, 9, 235–237.

HANEEF S S, SWARUP D, KALICHARAN and DWIVEDI S K (1995), 'The effect of concurrent lead and cadmium exposure on cell mediated immune response in goats', *Vet Human Toxicol*, 37, 428–429.

HATCH R C (1988), 'Poisons causing nervous stimulation or depression', in: Broth N H and Macdonald L (eds), *Veterinary Pharmacology and Therapeutics*, 6th edn, Iowa State University Press, Ames, IA, pp. 1083–1091.

HENRY S H, WHITAKER T, RABBANI I, BOWERS J, PARK D, PRICE W, BOSCH F X, PENNINGTON J, VERGER P, YOSHIZAWA T, VAN EGMOND H P, JONKER M and COKER R (2001), 'Aflatoxin M: safety evaluation of certain mycotoxin in food', *56th Meeting of JECFA, FAO Food and Nutrition Paper 74*, FAO, Rome.

HERNANDEZ L M, FERNANDEZ M A, JIMENEZ B, GONZALEZ J and GARCIA J F (1994), 'Organochlorine pollutants in meats and cow's milk from Madrid (Spain)', *Bull Environ Contam Toxicol*, 52, 246–253.

IDF (1991), *Monograph on Residues and Contaminants in Milk and Milk Products*, IDF-9101, International Dairy Federation, Brussels.

IDF (1997), *Monograph on Residues and Contaminants in Milk and Milk Products*, IDF-9701, International Dairy Federation, Brussels.

IVEY M C, DEHLER D D and CLABONN H W (1993), 'Gas liquid chromatography determination of chlorfenvinphos in milk, egg and body tissues of cattle and chicken', *J Agric Food Chem*, 41, 822–824.

JADHAV G D (1986), 'DDT and BHC residues in milk samples from Marathwada region', in: Goel S C (ed.), *Pesticide Residues and Environmental Pollution*, National Symposium, Muzaffarnagar, India, pp. 86–92.

KALANTZI O I, ALCOCK R E, JOHNSTON P A, SANTILLO D, STRINGER R L, THOMAS G O and JONES K C (2001), 'The global distribution of PCBs and organochlorine pesticides in butter' *Environ Sci Technol*, 35, 1013–1018.

KALPANA B (1999), 'Human health risk assessment for exposures to pesticides: a case study of endocrine disruptors', *Proc Eighth Nat Symp on Environment*, Kalapakkam, India, pp. 70–72.

KALRA R L and CHAWLA R P (1981), 'Impact of pesticidal pollution in the environment', *J Bombay Nat Hist Soc*, 78, 1–15.

KALRA R L and CHAWLA R P (1985), 'Pesticidal contamination of foods in the year 2000 A.D.', *Proc Ind Natl Sci Acad*, B52, 188–204.

KALRA R L, CHAWLA R P, SHARMA M L, BATTU R S and GUPTA S C (1983), 'Residues of DDT and HCH in butter and ghee in India, 1978–1981', *Environ Pollut Ser B*, 6, 195–206.

KALRA R L, KAUR H, SHARMA S, KAPOOR S K, KSHIRASAGAR R B, VAIDYA R C, SAGADE R B, SHIROLKAR S B, DIKSHIT S S, RAIZADA R B, SRIVASTAVA M K, APPAIAH K M, SRINIVASA M A, USHA RANI M, RAMA RAO S N, TOTEZA G S, DASGUPTA J and GHOSH P K (1999) 'DDT and HCH residues in dairy milk samples collected from different geographical regions of India – a multicentric study', *Food Addit Contam* 16, 411–417.

KALRA R L, KAUR H, SHARMA S and KAPOOR S K (2001), 'Surveillance of DDT and HCH residues in infant formula samples and their implication on dietary exposure: a multicenter study', *Pestic Res J*, 13, 147–151.

KANNAN K (1994), 'Food pollution by organochlorine and organotin compounds in tropical Asia and Oceania', Ph D thesis, Ehime University, Matsuyama, Japan.

KANNAN K, TANABE S, RAMESH A, SUBRAMANIAN A and TATSUKAWA R (1992), 'Persistent organochlorine residues in foodstuffs from India and their implications on human dietary exposure', *J Agric Food Chem*, 40, 518–524.

KANNAN K, TANABE S, GIESY J P and TATSUKAWA R (1997), 'Organochlorine pesticides and polychlorinated biphenyls in foodstuffs from Asian and Oceanic countries', *Rev Environ Contam Toxicol*, 152, 1–55.

KAPHALIA B S, TAKROO R, MEHROTRA S, NIGAM U and SETH T D (1990), 'Organochlorine pesticide residues in different Indian cereals, spices, vegetables, fruits, milk, butter, desi ghee and edible oils', *J Assoc Off Anal Chem*, 73, 509–512.

KAPOOR S K, CHAWLA R P and KALRA R L (1980), 'Contamination of bovine milk with DDT and HCH residues in relation to their usage in malaria control programme', *J Env Sci Hlth*, B(15), 545–557.

KATHPAL T S, SINGH G, YADAV G S, DHANKAR J S and SINGH A (1992), 'Monitoring of milk and milk products for DDT and HCH contamination', *Pestic Res J*, 4, 123–131.

KATHPAL T S, KUMARI B, SINGH S and SINGH J (2004) 'Multiresidue analysis of bovine milk and human milk in cotton growing belt of Haryana', in: Dureja P, Saxena D B, Kumar J, Gopal M, Singh S B and Tanwar R S (eds), *Pesticide Environment and Food Security*, Society of Pesticide Science India, New Delhi, pp. 140–148.

KHANDEKAR S S, NORONHA A B C and BANERJEE S A (1981), 'Organochlorine pesticide residues in eggs and milk available in Bombay markets', *Sci Cult*, 47, 137–139.

KHASKHELI M, MALIK R S, ARAIN M A, SOOMRO A H and ARAIN H H (2008), 'Detection of β-lactam antibiotic residues in market milk', *Pak J Nutr*, 7, 682–685.

KITUYI E N, WANDIGA S O and JUMBA I O (1997), 'Occurrence of chlorfenvinfos residues in cow's milk sampled at a range of sites in Western Kenya', *Bull Environ Contam Toxicol*, 58, 969–975.

KOCH H and VARENHOLT F (1981), *Seveso ist Uberall*, Fischer Verlag, Köln, Germany.

KOLACZ R, GORECKA H and DOBRZANSKI Z (1996), 'Mercury and selenium concentration in cow milk from regions of ecological degradations', *Bromatologia i Chemia Toksykologiczna*, 29, 225–228.

KUMAR A, DAYAL P, SINGH G, PRASAD F M and JOSEPH P E (2005), 'Persistent organochlorine pesticide residues in milk and butter in Agra city, India: a case study', *Bull Environ Contam Toxicol*, 75, 175–179.

KUMAR N (2009), 'Monitoring of veterinary drug residues in milk and milk products: present scenario in India', in: Singh S P, Funk J, Tripathi S C and Joshi N (eds), *Food Safety Quality Assurance and Global Trade – Concerns and Strategies*, International Book Distributing Co., Lucknow, India, pp. 219–226.

KUMAR N R and NATH A (1996) 'Monitoring of bovine milk for DDT and HCH', Pestic Res J, 8, 90–92.

KUMAR N R, NATH A and BHALLA O P (1992), 'Organochlorine insecticide residues in commercial brands of baby milk powder', *Pestic Res J*, 4, 51–53.

LAKSHMINARAYANA V and MENON P K (1975), 'Screening of Hyderabad market samples of foodstuffs for organochlorine insecticide residues', *Ind J Pl Prot*, 3, 14–19.

LATA S, SIDDIQUI M K J and SETH T D (1984), 'Chlorinated pesticides in desi ghee', *J Food Sci Tech*, 21, 94–95.

LI C F, BRADLEY R L JR and SCHULTZ I H (1970), 'Fate of organochlorine pesticides during processing of milk into dairy products', *J Assoc Off Anal Chem*, 53, 127–138.

LINO C M and NORONHA DA SILVEIRA M I (1992), 'Organophosphorus pesticide residues in cow's milk: levels of *cis*-mevinphos, methyl-parathion and paraoxon', *Bull Environ Contam Toxicol*, 49, 211–216.

LITWINCZUK A, DROZD-JANCZAK A, PIEROG M and DOROSZ D (1999), 'Lead and cadmium content in milk produced in the vicinity of Bogdanka coal mine', *Medycyna Weterynaryjna*, 55, 757–759 (in Polish).

MATHUR M P, DATTA ROY D and DINAKAR P (1999), in: *Text Book of Dairy Chemistry*, DIPA, ICAR, New Delhi, pp. 394–396.

MAZARIEGOS F (1976), 'An environmental economic study of the consequences of pesticide use in Central American cotton production', in: *Impact Monitoring of Agricultural Pesticides*, United Nations Programme, AGP 1976/M/4, Rome, pp. 15–18.

MCEWEN S A, BLACK W D and MEEK A H (1992), 'Antibiotic residues (bacterial inhibitory substances) in the milk of cows treated under label and extra label conditions', *Can Vet J*, 33, 527–534.

MERAI M and BOGHRA V R (2004), 'Chemical pollutants – their occurrence, sources of contamination in milk and milk products and methods of removal – a review', *Ind J Dairy Sci*, 57, 291–305.

MITRA N G, UPADHYAY A, SACHIDANAND B and AGARWAL G D (1999), 'Contamination of pesticides in samples of milk and milk products', *Pestology*, 23, 36–40.

MUKHERJEE I and GOPAL M (1993), 'Organochlorine pesticide residues in dairy milk in and around Delhi' *J AOAC International*, 76, 283–286.

NAG S K and RAIKWAR M K (2006), Final Report of Lal Bahadur Shastri Young Scientist Scheme: 'Monitoring of pesticide residues in animal feed, fodder and milk with special reference to Bundelkhand region', submitted to ICAR, New Delhi.

NAG S K and RAIKWAR M K (2008) 'Organochlorine pesticide residues in bovine milk', *Bull Environ Contam Toxicol*, 80, 5–9.

NAG S K, MAHANTA S K, RAIKWAR M K and BHADORIA B K (2007), 'Residues in milk and production performance of goats following the intake of a pesticide (endosulfan)', *Small Ruminant Research*, 67, 235–242.

NASR I N, SALLAM A A A and ABD EL-KHAIR A A (2007), 'Monitoring of certain pesticide residues and some heavy metals in fresh cow's milk at Gharbia Governorate, Egypt', *J Appl Sci*, 7, 3038–3044.

NIGAM U and SIDDIQUI M K J (2001), 'Organochlorine insecticide residues in dairy milk samples collected in Lucknow, India', *Bull Environ Contam Toxicol*, 66, 678–682.

NIZAMLIOGLU F, AKTUMSEK A, KARA H and DINC I (2005), 'Monitoring of some organo-chlorine pesticide residues of butter in Konya, Turkey', *J Environ Biol*, 26 (2 suppl), 375–378.

OKADA I A, SAKUMA A M, MAIO F D, DOVIDAUS S and ZENEBON O (1997), 'Evaluation of lead and cadmium level in milk due to environmental contamination in the Paraiba Valley region of South-Eastern Brazil', *Revista de Saude Publica*, 31, 140–143.

OZDEMIR S, DUNDAR M S, SENGORUR B and SENOL A S (2009), 'Lead and zinc content of cows' milk affected by varying traffic density', *Int J Env Pollut*, 36(4), 411–417.

PAGLIUCA G, SERRAINO A, GAZZOTTI T, ZIRONI E, BORSARI A and ROSMINI R (2006), 'Organophosphorus pesticide residues in Italian raw milk', *J Dairy Res*, 73(3), 340–344.

PANDIT G G, SHARMA S, SRIVASTAVA P K and SAHU S K (2002), 'Persistent organochlorine

pesticide residues in milk and dairy products in India', *Food Addit Contam*, 19, 153–157.

PARDIO V T, WALISZEWSKI K N, LANDIN L A and BAUTISTA R G (2003), 'Organochlorine pesticide residue in cow's milk from a tropical region of Mexico', *Food Addit Contam*, 20, 259–269.

PATEL P M, NETKE S P, GUPTA B S and DABADGHAO A K (1981), 'Note on the survey of consumer milk supplied to Jabalpur city for the incidence of aflatoxin M_1 and M_2', *Ind J Anim Sci*, 51, 906.

PAUL R, KALRA M S and SINGH A (1976), 'Incidence of aflatoxins in milk and milk products', *Ind J Dairy Sci*, 29, 318–321.

PRACHAR V, VENINGEROVA M, UHANK J and PRIBELA A (1995),'Persistent organochlorine compounds in cow's milk and butter', *Fresenius Environ Bull*, 4, 413–417.

PRIYO B V and CONTIN-ESNAULT D (1996), 'A survey of nitrate contents in Indonesian milk by enzymatic analysis', *Food Addit Contam*, 13, 77–87.

RAJAN A, ISMAIL P K and RADHAKRISHNAN V (1995), 'Survey of milk samples for aflatoxin M_1 in Thrissur, Kerala', *Ind J Dairy Sci*, 48, 302–305.

RANA T, SARKAR S, MANDAL T K, BHATTACHARYYA K, ROY A and KOL L (2008), 'Contribution of arsenic from agricultural food chain in Nadia District of West Bengal in India', *Internet Journal of Thoracic and Cardiovascular Surgery*, 4(2).

RIVA C and ANADAN A (1991), 'Organochlorine pesticides in cow's milk from agricultural regions of Northwestern Spain', *Bull Environ Contam Toxicol*, 46, 527–533.

SAAD A M, ABDELGADIR A M and MOSS M O (1989), 'Aflatoxin in human and camel milk in Abu Dhabi, United Arab Emirates', *Mycotox Res*, 5, 57–60.

SALAS J H, GONZALEZ M M, NOA M, PEREZ N A, DIAZ G, GUTIERREZ R, ZAZUETA H and OSUNA I (2003), 'Organophosphate pesticide residues in Mexican commercial pasteurized milk', *J Agric Food Chem*, 51, 4468–4471.

SANG S, PETROVIC S and CUDDEFORD V (1999), *Lindane: A Review of Toxicity and Environmental Fate*, Wildlife Toxicology Programme, Toronto, Canada.

SAXENA M C and SIDDIQUI M K J (1982), 'Pesticide pollution in India: Organochlorine pesticides in women, buffalo and goat milk', *J Dairy Sci*, 65, 430–434.

SHAH P G, PATEL B K, RAJ M F and PATANWADIA R D (1992), 'Monitoring of HCH and DDT residues in ghee and butter samples in Gujarat', *Pestic Res J*, 4, 117–121.

SHARMA H R, KAUSHIK A and KAUSHIK C P (2007), 'Pesticide residues in bovine milk from a predominantly agricultural state of Haryana, India', *Environ Monit Assess*, 129, 349–357.

SHASTRY M S (1983), 'Monitoring of pesticide residues in animal feeds and animal products', *Pesticides* 10, 36–38.

SHEMAKAYANI S B and PATEL G S (1989), *Arogya*, 15, 32.

SIMSEK O, GULTEKIN R, OKSUZ O and KURULTAY S (2000), 'The effect of environmental pollution on the heavy metal content of raw milk', *Nahrung*, 44, 360–363.

SINGH B B and KUMAR V (1996), 'Lead contamination of milk from Gorakhpur district', *J Living World*, 3, 36–39.

SINGH P P, BATTU R S, JOIA B S, CHAWLA R P and KALRA R L (1986), 'Contribution of DDT and HCH used in malaria control programme towards the contamination of bovine milk', in: Goel S C (ed.), *Pesticide Residues and Environmental Pollution*, National Symposium, Muzaffarnagar, India, pp. 86–92.

SINGH R B, SAHA R C and MISRA R K (2002), 'Arsenic profile in livestock feeds and products in Ghentuga and Gontra district of West Bengal', Project report of the work done at Eastern Regional Station of NDRI, Kalyani-741235, in: Chhabra A (2004),

'Contaminants in milk and milk products and measures to minimize their levels', *Indian Dairyman*, 56, 115–125.

SIROTKIN A N, VORONOV S I, RASIN I M, KORNEEV N A, SOKOLOVA E A, SIDOROVA E V, ISAMOV N N JR and TSEITIN K F (2000), 'Migration of heavy metals in the trophic chain of lactating cows in the Moscow region', *Russ Ag Sci*, Publ 2001, 41–47, translated from *Doklady Rossiiskoi Akademii Sel'skokhozyaistvennykh Nauk* (2000), No. 4, 37–39.

STEINWANDTER H (1978), 'Experiments on lindane metabolism in plants. III. Formation of beta-HCH', *Bull Environ Contam Toxicol*, 20, 174–179.

STEINWANDTER H and SCHLÜTER H (1978), 'Experiments on lindane metabolism in plants. IV. A kinetic investigation', *Bull Environ Contam Toxicol*, 20, 174–179.

STEVENS J B (1991), 'Disposition of toxic metals in the agricultural food chain. 1. Steady-state bovine milk biotransfer factors', *Env Sci Tech*, 25, 1289–1294.

SUDARSHAN R V and BHAT R V (1995), 'A survey on veterinary drug use and residues in milk in Hyderabad', *Food Addit Contam*, 12, 615–620.

SUHREN G, HOFFMEISTER A, REICHMUCH J and HEESCHEN W (1990), 'Incidence of inhibitory substances in milk for consumption from various European countries', *Milchwissenschaft*, 45, 485–490.

SURENDRA NATH B, UNNIKRISHNAN V, PREEJA C N and RAMA MURTHY M K (2000), 'A study on the transfer of organochlorine pesticide residues from the feed of the cattle into their milk', *Pestic Res J*, 12, 58–73.

SURENDRA NATH B, USHA M A, SARWAR and UNNIKRISHNAN V (2005), 'Organochlorine pesticide residues in milk from South India', *Ind J Dairy Sci*, 58, 247–249.

SWARUP D, DWIVEDI S K and DEY S (1997), 'Lead and cadmium levels in blood and milk of cows from Kanpur city', *Ind J Anim Sci*, 67, 222–223.

SWARUP D, PATRA R C, NARESH R, KUMAR P and SHEKHAR P (2004), 'Blood lead levels in lactating cows reared around polluted localities; transfer of lead into milk', *Sci Total Environ*, 347, 106–110.

SZERLETICS T M, SOOS K and VEGH E (2000), 'Determination of residues of pyrethroid and organophosphorus ectoparasiticides in foods of animal origin', *Acta Vet Hung*, 48, 139–149.

TANABE H (1972), 'Contamination of milk with chlorinated hydrocarbon pesticides', in: Matsumura F, Bousch G M and Misato T (eds), *Environmental Toxicology of Pesticides*, Academic Press, New York, pp. 239–256.

TIWARI V and CHAUHAN R K S (1991), 'Aflatoxin detection in milk samples of cattle', *Natl Acad Sci Lett*, 14, 391–392.

TOMIZAWA C (1977), 'Past and present status of residues of pesticides marketed in Japan', *Japan Pestic Inf*, 30, 5–42.

TOPCU A, TOPCU A A, SALDAMLI I and YURTTAGUL M (2006), 'Determination of nitrate and nitrite content of Turkish cheeses', *African J Biotech*, 5(15), 1411–1414.

TRIPATHI H C (1966), 'Organochlorine insecticide residues in agricultural and animal products in Terai area', M.Sc. Thesis, Uttar Pradesh Agricultural University, Pantnagar, India.

UNNIKRISHNAN V, SURENDRA NATH B, USHA M A, SARWAR and BHAVADASAN, M K (1999), 'Organochlorine pesticide residues in milk and milk products', *Ind J Dairy Biosci*, 10, 33–37.

UYETA M, TANE S and NISHIMOTO T (1970), 'Residues of BHC isomers and other organochlorine pesticides in fatty foods of Japan', *J Fd Hyg Soc*, 11, 256–263.

VAIDYA P S, THANKARAJ M T and JASWAL P K (1998), 'Assessment of aflatoxin M1 in selected butter samples in Nagpur city', *Ind Dairyman*, 50, 21–23.

VASANTHI D, KUTTLAM S, JAYAKUMAR R, VIJAYALAKSHMI K and CHANDRASEKARAN S (2003), 'Monitoring of bovine milk samples for chlorinated pesticides', Pestic Res J, 15, 193–194.

VEIEROV D, AHARONSON N and ALUMOS E (1977), 'Residues of HCH isomers and DDT derivatives in Israel milk and seasonal fluctuation', *Phytoparasitica*, 5, 26–33.

VENANT A, BORREL S and MALLET J (1991), 'Organochlorine compounds contamination in milk and dairy products from 1975–1989', *Lait*, 71, 107–116.

WALISZEWSKI S M (1993a), 'Residues of lindane, HCH isomers and HCB in the soil after Lindane application', *Environ Pollut*, 82, 289–293.

WALISZEWSKI S M (1993b), 'Conversion of lindane to HCH isomers and HCB in the agricultural field conditions', in: Mansour M (ed.), *Fate and Prediction of Environmental Chemicals in Soils, Plants, and Aquatic Systems*, Lewis Publishers, Boca Raton, FL, pp. 149–157.

WALISZEWSKI S M (1995), 'Residues of HCH isomers and HCB in root vegetables after the application of lindane to the soil', *Revista Internacional de Contaminacion Ambientai*, 11, 13–19.

WALISZEWSKI S M, PARDIO V T, WALISZEWSKI K N, CHANTIRI J N, INFANZON R M and RIVERA J (1996), 'Detection of some organochlorine pesticides in cow's milk', *Food Addit Contam*, 13, 231–235.

WALISZEWSKI S M, VILLALOBOS-PIETRINI R, GOMEZ-ARROYO S and INFANZON R M (2003), 'Persistent organochlorine pesticide levels in cow's milk samples from tropical regions of Mexico', Food Addit Contam, 20, 270–275.

WHO (1992), 'Hexachlorocyclohexanes', *Health and Safety Guide*, Environmental Health Criteria 123, World Health Organisation, Geneva.

WONG S K and LEE W O (1997), 'Survey of organochlorine pesticide residues in milk in Hong-Kong (1993–1995)', *J Assoc Off Anal Chem Int*, 80, 1332–1335.

YAMAKI M, BERRUGA M I, ALTHAUS R L, MOLINA M P and MOLINA A (2004), 'Occurrence of antibiotic residues in milk from Manchega Ewe dairy farms', *J Dairy Sci*, 87, 3132–3137.

YOUSEF A E and MARTH E H (1985), 'Degradation of aflatoxin M-1 in milk by UV energy', *J Food Prot*, 48, 697–698.

ZHANG Y (1995), 'Levels of organochlorine pesticide residues in food of China', *Pest Mgmt Sci*, 6, 20–22.

ZHONG W, XU D, CHAI Z and MAO X (2003), '2001 Survey of organochlorine pesticide in retail milk from Beijing, P.R. China', *Food Addit Contam*, 20, 254–258.

ZMUDZKI J, JUSZKIEWICZ T, NIEWIADOWSKA A, SZKODA J, SEMENIUK S, GOLEBIOWSKI A and SZYPOSZYNSKI K (1992), 'Chemical pollution of tissues from cattle and in milk and eggs in the Zgorzelec-Bogatynia district', *Medycyna-Weterynaryjna*, 48, 213–215.

6

Contaminants in milk: routes of contamination, analytical techniques and methods of control

S. K. Nag, Indian Grassland and Fodder Research Institute, India

Abstract: This chapter deals with the various sources of chemical contamination, their analytical techniques, regulatory aspects and methods to manage the contaminants. The chapter also provides the maximum legal limits of pesticide residues, veterinary drugs, heavy metals and aflatoxin as set by different agencies.

Key words: contaminants, milk, milk products, pesticide residues, heavy metals, radionuclides, aflatoxins, mycotoxins, veterinary drugs, antibiotics, nitrates, detergents, disinfectants, analytical techniques, MRL, regulatory aspects.

6.1   Introduction

In the previous chapter the diverse types of chemical contaminants, including pesticide residues, heavy metals, radionuclides, veterinary drugs and antibiotics, mycotoxins, nitrates and nitrites, which could be present in milk and milk products and their adverse effect on human and animal health were discussed. The status of such contaminants in milk from different geographical locations was also reviewed. In the present chapter, the specific sources by which the chemical contaminants enter the animal body and subsequently contaminate milk and its products or directly contaminate them are highlighted. The laboratory analytical techniques to detect and measure the contaminants at ultra-low level with the latest developments in the field have also been discussed. The worldwide concern regarding the presence of toxicants in milk has led various

national and international agencies to formulate guidelines in order to regulate the management of contaminants so that their presence is below the prescribed limits. So, an effort has also been made in this chapter to touch upon the regulatory aspects and possible management techniques that can be adopted for the production of safe and quality milk.

6.2   Sources of contamination

Pesticides can enter the animal body in three possible ways (Fig. 6.1):

- From contaminated feed and fodder
- From contaminated water
- Through skin pores when the animal is sprayed or drenched to treat ectoparasites.

The main source of entry is contaminated feed and fodder offered to animals for consumption. The feed materials may be contaminated by pesticides at the time of cultivation of the crops, from which they have been prepared or during the storage of the feed materials, grains, seeds, etc., in, for example, warehouses and other places where pesticides are used to keep away pests and plant pathogens.

Green fodders and/or dual-purpose crops (such as sorghum, maize, bajra, oat, berseem, cowpea and different grasses) grown in the field are often sprayed with pesticides to save them from the onslaughts of diseases and pests. This may act as a direct source of contamination because on many occasions fodder crops after being treated with pesticides are fed directly to animals without waiting for a sufficient time to allow the residues to decrease to levels below the maximum residue limit. Pesticides are also used on a large scale to kill ectoparasites like ticks, mites and insects on the animal body. As a result they may enter into the animal body through skin pores when applied by drenching. Water sometimes

Fig. 6.1   Possible sources of contamination of animal body and animal products with pesticides.

may also be a source of contamination, although concentration may not be such as to worry about. After entry into the animal body pesticides are distributed in different tissues and organs. Since many of them, particularly organochlorines and synthetic pyrethroids, are lipophilic in nature, they have a tendency to accumulate in the adipose tissues and usually they do not get converted into water-soluble metabolites. As the concentration in the fatty tissues exceeds a threshold level, the excess residues are translocated into the lactating glands of the animals from where they are secreted in the milk. Because of the special position of milk in the diet of infants and children, the presence of residues in milk is undesirable. That is why the residue limits for milk and dairy products tend to be more severe than those for other products. Numerous examples of the contamination of animals through animal feed have been reported in the literature (Buck, 1970, 1975; Van Houweling *et al.*, 1977; Pierson *et al.*, 1982; Kaphalia and Seth, 1982; Shastry, 1983; Singh *et al.*, 1988, 1997; Dikshith *et al.*, 1989a,b; Battu *et al.*, 1996; Unnikrishnan *et al.*, 1998; Agnihotri, 1999; Kang *et al.*, 2002; Sharma *et al.*, 2005; Nag and Raikwar, 2006).

The presence of polychlorinated biphenyls (PCB) in milk has been traced back to cattle feed and other sources such as silos coated with sealant or paint, oils and grease containing PCBs, and sewage and sludge application to agricultural pasture. The main suspected sources of PCBs are electrical appliances like transformers and capacitors. PCBs are also released in the environment from ship-breaking activities, from different kinds of weapons, aircraft, tanks and explosives used by the army during training or war.

Dioxins and dibenzofurans are highly persistent chemicals with average half-life of around 10 years. They are formed as unwanted by-products from many different industrial processes such as metal smelting, chlorine bleaching of paper pulp, manufacture of some herbicides, burning of solid materials like coal, straw, waste or biosludge, and natural processes like volcanic eruptions and forest fires. These compounds ultimately get deposited in soil through dumping of industrial wastes. From soil and atmosphere they reach forages and grasses. The main route of dioxin in milk is through forage fed to dairy animals. They may enter through contamination of grass with smoke particles. The carryover rates vary from 0 to 40% depending upon the type of dioxin/furan. The equilibrium between uptake and release with the milk fat is reached 40–80 days after the beginning of a continuous exposure to dioxins/furans.

Special attention has been paid during the last decade to heavy metal contamination in the environment and its impact on humans and animals. As with pesticides, heavy metal contamination may also originate through ingestion of contaminated feed, fodder and/or drinking water by animals. Industrial effluents, waste chemicals, municipal sewage and sludge and inorganic fertilisers can contaminate water and soil with toxic metal residues. Irrigation water, if not properly treated, carries with it all the pollutants, which ultimately are deposited in the soil. Soils actually act as a reservoir of the toxic metals. Combustion of fossil fuel and emission of industrial smoke pollute the atmosphere and surrounding environment and release heavy metals, which ultimately get deposited

on nearby crops and soil. From soil, many crop plant species take up those heavy metals along with other essential nutrients and deposit them in their tissues. Rarely, mineral supplements containing some heavy metals in higher proportion may also act as a source of contamination, especially when given at a high dose. Once in the animal system these metals can persist for several weeks even after discontinuation of the exposure. From tissues they may enter lactating glands through physiological processes and may be secreted through milk. Heavy metal contamination in milk and its products may also originate during transportation, processing and storage in metallic containers, through use of food additives and through atmospheric pollution. The secretory route is the principal route through which heavy metals contaminate milk, whereas the post-secretory route has limited importance but may occur when milk comes into contact with unsuitable surfaces or equipment, particularly when pH is lowered through the fermentation processes.

All the antimicrobial drugs administered to cows can enter the milk to some degree and for a certain period of time. Antibiotic residues can also enter the milk supply when treated cows are returned to the milking herd early, i.e. before the expiry of the withholding period or when a cow retains the antibiotic residues in her system for an extraordinary length of time. The residue can be the drug itself or its metabolites. In every case when the diseased animal is treated directly, either orally, parenterally or by cutaneous application, by spraying, dusting or pour-on application of the veterinary remedy, this leads to an immediate contamination of the prospective food component of the treated animal within just a few minutes. The transfer of residues into milk, if any, originates from absorbed parts of the dose passing the blood–milk barrier in the mammary gland. The antibiotic residues might appear in milk from various sources such as treatment of mastitis and other diseases, injectables, or through feed contaminated with antibiotics (Carlsson and Bjorck, 1991). The dominating residues in most countries are β-lactam antibiotics and sulfa drugs but others, e.g. tetracyclines, aminoglycosides and chloramphenicol, also occur. Many hormones such as anabolic steroids and β-agonists, which are being used illegally to promote growth of animals, find their way into the milk. But, nevertheless, the level of antibiotic residue in milk remains very low and human health risks associated with these residues are small as compared to other contaminants. Sub-therapeutic use of these drugs through food and water does not generally result in residues in milk. All the drugs have a definite withdrawal period, which, if followed, results in no risk of contamination of milk.

The principal source of mycotoxin in milk and milk products is contaminated feed and fodder. Feed and fodder quite often get contaminated with various toxin-producing fungi while growing in the field or during transportation and storage. Mycotoxin contamination in animal feed because of poor storage is a major problem in many countries. Mishra and Singh (1978) observed that 18 out of 36 samples collected from a flood-affected area of Mathura in India were positive for aflatoxin (AF). About 70% of the groundnut cake samples sold in

Hapur market in Uttar Pradesh in India were contaminated with AFB1 in the range of 113–2250 ppb (Rampal *et al.*, 1979). A survey conducted by Patel *et al.* (1981) revealed that about 64% and 80% of samples of groundnut and cottonseed were contaminated with AFB1, respectively. About 25% of the positive samples of groundnut and cottonseed contained AFB1 between 0.2 and 0.52 ppm. More than 50% of the samples of sorghum, maize and wheat exhibited AFB1 contamination. Balaraman and Gupta (1990) found 50% of maize, 30% of rice bran, 25% of millet wastes and 13% of mustard cake samples from Sikkim in India positive for aflatoxin. Out of 56 samples of various feed ingredients collected from Karnal, India, 46 samples were found to contain AFB1 between 20 and 4200 ppb (Prasad *et al.*, 1997). A high level of aflatoxins in groundnut cake, maize and compound feed was also reported from southern India by Gowda *et al.* (2003). When a milk animal consumes a feed contaminated with AFB1, the toxin is carried over into the milk as AFM1, which can be detected in milk 12–24 h after the first ingestion of AFB1. The carryover rate of AFB1 from feed to AFM1 in milk, which varies from 1.5% to 4.0% (van Egmond, 1989; Borkhatriya *et al.*, 2001), is influenced by several factors, viz., concentration of AFB1 in feed, duration of feeding of contaminated ration, season of the year, individuality of the animal, species of animal, stage of lactation and milk yield. The toxin becomes undetectable two weeks after withdrawal of contaminated feed. Some milk products like cheese, paneer, etc., can be contaminated with different moulds which may produce AFB1, thereby directly contaminating the products. Polan *et al.* (1974) showed that AFM1 can be expected to appear in milk when AFB1 in concentrated feed exceeds 46 ppb.

The main source of nitrate and nitrite contamination in milk is the feeding of forages grown in high nitrate-containing soil as a result of application of a large amount of nitrogen-containing fertilisers. Nitrates may also come from irrigation and drinking water. Milk and milk products get contaminated with nitrite, nitrate and nitrosamines either through the secretary or post-secretary route, or with endogenous and exogenous formation of nitrite and nitrosamines. Only nitrate has a significant post-secretary source as it is permitted in some countries as an additive to milk to prevent late gas defect in cheese.

Detergents and disinfectants can contaminate milk and milk products if proper care is not taken when rinsing the udder, utensils and installations and not allowing proper drainage after the use of these chemicals.

6.3   Analytical techniques

6.3.1   Pesticide residues and persistent organic pollutants (POP)

Pesticide residue analysis, like any other chemical analysis, consists of sequential steps: sampling, extraction of pesticides from the sample matrix, clean-up of the extracts and finally identification and quantification.

Sampling, i.e. selection and collection of aliquots, should be done in such a way that it truly represents the whole lot, otherwise the analytical results would

not be meaningful. Usually sampling is done in a randomised manner, particularly in the case of residue monitoring in environmental samples. For milk samples, transportation and storage is very critical because of milk's highly perishable nature. Milk fat is of lower density than other constituents and tends to rise to the surface. Thorough mixing of milk with a proper instrument that will reach the entire depth of the liquid is essential. Milk churns easily at 26.5–29.5°C and agitation near this temperature should be avoided. Milk samples should be collected in glass bottles and stored in cool, insulated containers while transporting from the place of collection to the laboratory for analysis. In the laboratory, milk samples should be stored in a refrigerator to avoid spoilage. Samples can also be preserved with formalin (0.1 ml in 25 ml milk) or potassium dichromate (0.06 g per 100 ml). Samples after collection may also be mixed with amyl alcohol (1 ml of 1%) in a saturated aqueous solution of potassium permanganate in 200 ml of milk.

Pesticides can be extracted from milk by employing different techniques such as blending with organic solvents such as hexane, or hexane and acetone, or diethyl ether (Luke and Doose, 1984), solvent partitioning (Toyoda *et al.*, 1990), blending followed by centrifugation (Kapoor *et al.*, 1981) and single column extraction and clean-up (Stijve, 1983). For highly lipophilic organochlorines sometimes only milk fat is extracted from milk and then residues are determined as accumulated in the extracted fat. But residues may also be present in the non-fat portion of milk, particularly in the case of pesticides such as OPs, carbamates, etc. In these cases, the whole milk is extracted and residues are estimated in whole milk. Moreover, additional clean-up is required whenever residues are estimated in isolated fats and oils, which itself presents a challenging task to the residue chemist. This is because the final extract may contain enough fat to interfere with gas liquid chromatographic (GLC) analysis.

After extraction, samples are subjected to various clean-up techniques to separate the pesticide compounds from the substrate matrix, coextractives and impurities because otherwise they would interfere with the estimation. At the beginning of residue analysis work in milk, which involves extraction of fat first, several clean-up techniques have been developed to successively remove the interference by fats in the final analysis as much as possible. Eidelman (1962) used dimethyl sulfoxide after isolation of fat, which took 6 hours per sample and could be dangerous under certain conditions. McCully and McKinley (1964) reported a lengthy low-temperature fat-precipitating method unsuited for routine use. Rogers (1972) investigated the use of Micro Cel-E, an adsorbent, to remove large quantities of extracted fat from residues. This method was better than the previous ones. Griffith and Craun (1974) employed gel permeation chromatography to remove fats from extracts. Pesticides were also separated from fat by column chromatography on florisil (Suzuki *et al.*, 1979; Stimac, 1979), alumina (Telling and Sisons, 1977; Luke and Doose, 1984), silica (Johnson *et al.*, 1976; Specht and Tilkes, 1980), by sweep-codistillation (Heath and Black, 1979, 1980) or by extraction with concentrated sulfuric acid (Veierov and Aharonson, 1978, 1980, and many others).

Most of the monitoring work on pesticide residues in milk targeted only DDTs and HCHs and the conventional sulfuric acid partitioning clean-up technique was used. But sulfuric acid clean-up is suitable only for acid-stable organochlorines (OC) like HCH isomers and DDT compounds, not for other OCs, organophosphates (OP), carbamates, synthetic pyrethroids (SP) and others. Solvent partitioning followed by column clean-up with florisil (synthetic magnesium silicate) is a suitable procedure for most of the pesticides and is widely used. Cleaned-up extracts are finally analysed by chromatographic techniques such as GLC or high performance liquid chromatography (HPLC) for identification and quantification.

When the target pesticide is any particular compound that is supposed to be present in milk due to its use in any feed, fodder or applied to the animal, or the intended study is to observe the passage of a particular compound from feed to milk in a controlled feeding experiment on animals, the analytical method is developed just to analyse the single compound, which may also include some of its isomers or metabolites. But in a monitoring programme for pesticide residues in any commodity, samples of which are collected without any proper knowledge about the presence of any particular compound, methodologies are developed targeting a number of pesticides that are likely to be present. This is known as a multi-residue method, which can be defined as any analytical method that measures residues of a number of targeted pesticides simultaneously in a sample at a time. When monitoring multi-residues in any sample, the identity of the peaks obtained by chromatographic separation needs to be confirmed to ensure the authenticity of the results. Confirmation of the identity of the compounds may be done by use of alternate columns having different polarity, relative retention time and derivatisation or more appropriately using GC-mass spectroscopy (GC-MS) or liquid chromatography-MS-MS (LC-MS-MS). Some of the multi-residue methods developed for milk are discussed below.

Mills *et al.* (1972) developed a method involving extraction of fats along with residues from milk in diethyl ether and hexane after denaturing the product with oxalic acid and alcohol. The ether extract containing residues was washed with water to remove water-soluble co-extractives and then partitioned between hexane and acetonitrile prior to clean-up in a florisil column before analysis by GLC. Kapoor *et al.* (1981) developed a simplified method for estimation of DDT and HCH residues in milk. This method involved blending of milk with acetone and hexane (1:1 v/v) for two minutes followed by centrifugation of the homogenate for 10 minutes at 2000 rpm. The cumulative upper hexane layer was concentrated and treated with concentrated sulfuric acid for clean-up. The upper hexane layer was washed with water to make it acid free, dehydrated and analysed by GLC. A very simple and rapid miniaturised method for monitoring of organochlorine pesticide residues in milk was developed by Stijve (1983). In this method 2 g of homogenised milk is mixed with florisil to make it a free-flowing powder, which is then extracted and cleaned up in a florisil column eluted with hexane and dichloromethane (DCM) in 4:1 v/v ratio. The eluate is concentrated

and care is taken to remove the traces of dichloromethane because otherwise it would interfere in analysis using ECD (electron capture detector) in GLC. The method is very sensitive, not only for OCs but also for OPs and SPs. This method was slightly modified by Battu *et al.* (2004) who used silica gel and anhydrous sodium sulfate in place of florisil to make 5 g of milk into a free-flowing powder, which was subsequently extracted and cleaned in a single column eluted with acetone and DCM (2:1 v/v). They did not use florisil in this method and recovery was more than 90% for all three groups, i.e. OC, OP and SP.

The method described by Tessari and Savage (1980) for estimation of OC residues in milk involved homogenisation with acetone–petroleum ether (1:2 v/v) followed by centrifugation at 2000 rpm for 10 minutes. The organic layer was subsequently washed with 2% sodium sulfate, dehydrated by passing through anhydrous sodium sulfate and subjected to column chromatography using florisil and sodium sulfate. Wong and Lee (1997) slightly modified this method by using gel permeation chromatography (GPC) as a clean-up procedure instead of solvent partitioning and obtained 86.8–104.1% recovery of DDT compounds, HCH isomers, dieldrin, heptachlor epoxide and HCB (hexachlorobenzene) from milk. The method used by Pardio *et al.* (2003), a modification of that proposed by Garrido-Frenich *et al.* (2000), involved extraction of fats from milk first and then an estimation of DDT and HCH residues accumulated in fat. Briefly, the milk fat was ground with anhydrous sodium sulfate and eluted with ethyl ether and hexane (1:1 v/v) in a glass column. The extract was centrifuged for 15 min at 3000 rpm. In the concentrated organic extract, 0.5 ml concentrated sulfuric acid was added and centrifuged for 10 min at 3000 rpm. The acid residue was again extracted with hexane, dried with a flow of nitrogen and cleaned in a glass column packed with anhydrous sodium sulfate. The column was eluted with 1% methanol in hexane. The dried extract, fortified with an internal standard of p,p'-dichlorobenzophenone prepared in hexane (10 mg/ml), was dissolved in hexane for GC analysis. Waliszewski and Szymczynski (1982) described a similar method for OC residue determination in fat extracted from milk. They isolated milk fat by centrifuging freeze-cooled milk, ground it with anhydrous sodium sulfate to make a coarse powder and then extracted it from a glass column with petroleum ether. The extract was cleaned up by sulfuric acid and analysed in GC. They did not use any further column clean-up and also no internal standard.

A rapid and sensitive method incorporating a simple extraction and clean-up procedure was developed for determination of malathion and malaoxon in milk and plasma by Muan and Skaree (1986). The compounds were analysed in GLC equipped with a phosphorus-selective detector and their identities were confirmed with GC-MS. Toyoda *et al.* (1990) developed a simple analytical method for six OP pesticides. The residues were extracted with acetonitrile added to milk, fat was removed by zinc acetate addition and dichloromethane partition and analytes after concentration were analysed by wide bore capillary GC. In their measurement of OP residues in milk, Salas *et al.* (2003) used a multi-residue method developed in the Netherlands. In this method milk is blended with ethyl acetate. Sodium sulfate is added and the mixture is shaken

and then allowed to stand for 2–3 minutes. The upper layer is decanted and a 50 ml aliquot is evaporated to dryness. The resulting residue is dissolved in hexane and extracted with acetonitrile saturated with hexane. The combined acetonitrile phase is evaporated, dissolved in ethyl acetate and subjected to GC analysis. The mean recovery rate of OPs such as dichlorvos, mevinphos, phorate, dimethoate, diazinon, disulfoton, methyl parathion, malathion, fenthion, chlorpyriphos, chlorfenvinphos and ethion varied from 43.3% (ethion) to 94.4% (chlorpyriphos).

For analysis of butter and ghee (clarified butter) samples should be first melted at 40–50ºC and decanted through a dry filter. A representative 3–5 g sub-sample, dissolved in hexane, is then extracted with acetonitrile saturated with hexane by partitioning. The acetonitrile phase is again partitioned with a dichloromethane and hexane mixture (15:85 v/v) in the presence of saturated sodium chloride solution. The combined organic layer is then dehydrated and concentrated. The concentrated extract may be dissolved in a hexane and acetone mixture (9:1 v/v) and analysed by GC for OPs. For further clean-up, the concentrated extracts are subjected to column chromatography with florisil. The column may be eluted with a hexane and dichloromethane mixture. The eluate after concentration is analysed in GLC for OCs and SPs.

The measurement of dioxins and PCBs in milk and milk products also involves the same procedures, i.e. extraction, clean-up in a florisil column to remove fats and subsequent analysis by chromatography. High resolution gas chromatography (HRGC) coupled with high-resolution mass spectrometry (HRMS) is currently the reference method used in the determination of very low concentrations of the common environmental contaminants like PCBs, PCDDs and PCDFs. The methods used to determine PCDDs and PCDFs in food must provide sufficient information to allow calculation of the results as toxic equivalents, at concentrations of 0.11 pg/g of fat in milk. For analysis of food samples with normal background contamination by polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) or PCBs, GC with high resolution MS has been validated in collaborative studies and has been shown to provide the required sensitivity and specificity.

6.3.2   Heavy metals and radionuclides

Heavy metals in milk can be analysed according to the method prescribed by AOAC (1990). In this case about 0.2 g of the sample is digested with a di-acid mixture consisting of concentrated nitric acid and concentrated sulfuric acid (2:1). The colourless extract is diluted with deionised water and analysed using atomic absorption spectrophotometry (AAS) or by ICP (inductively coupled plasma optical emission spectroscopy). For confirmation of the identity of the species in environmental samples, ICP coupled with mass spectroscopy (ICP-MS) is used. For digestion of samples, a microwave digestion apparatus can also be used instead of a hot plate or electrical heater for more efficient and environmentally friendly digestion. Anastasio *et al.* (2006) used the microwave system for acid digestion of sheep milk samples to be analysed for heavy metals.

Muñoz and Palmero (2004) described a sensitive and reliable technique for determination of trace amounts of Cd, Pb and Cu in milk using stripping potentiometry in a home-made flow cell. The optimum conditions for the method included an acetic acid–acetate buffer mixture (pH 3.4) as supporting electrolyte, an electrolysis potential of -1.1 V and a flow rate of 3 ml/min. A polarographic method was used by Valiukenaite *et al.* (2005) for analysis of the Pb and Cd content of milk. Ümit and Karayünlü (2008) developed a simple method for determination of Cd and Pb in milk serum prepared by spontaneous souring. Samples of raw milk were kept at room temperature (25ºC) for 48 h in order to allow the pH to decrease below 4.6 and the casein and fat to precipitate. The milk serum was filtered and directly measured in a graphite furnace AAS. The milk samples can also be assessed for heavy metal contamination electro-chemically based on advanced biosensor and immunosensor techniques. The use of adsorptive electrochemical analysis can reduce the need for sample preparation. In this case, the pretreatment of milk involves acid precipitation of proteins, followed by centrifugation and filtering to produce a clear filtrate for analysis.

Radionuclides present in milk samples may be analysed by ashing or isolation and extraction by complex formation and then detected by using a GM or scintillation counter. The radioisotopes ^{131}I, ^{140}Ba and ^{137}Cs can be analysed by gamma spectroscopy and ^{89}Sr by ion exchange or by chemical methods.

6.3.3   Veterinary drug residues

A capillary GC method was developed for the determination of seven penicillin residues in bovine tissue and milk (Meetschen and Petz, 1991). In this method, samples are extracted with acetonitrile under slightly acidic conditions and cleaned up by liquid–liquid partitioning and anion exchange chromatography. The penicillin residues were methylated with diazomethane and then analysed by GC. The recovery percentage varied from 65 to 80% and the limit of detection (LOD) was below 3 μg/kg. Liquid chromatographic methods have also been developed for the determination of penicillin residues in milk. A method utilising an automated clean-up procedure and isocratic chromatography with 0.01 M phosphate buffer – acetonitrile detected as little as 1 ng/ml penicillin with recoveries of 87–97% from spiked samples (Moats and Malisch, 1992). An ion-pairing LC method, which can determine four commonly used penicillins at the level of 3–4 ppb in milk, utilised tetrabutyl ammonium hydrogen sulfate to form ion pairs, which were then separated on an LC column using an aqueous acetonitrile mobile phase (Fletouris *et al.*, 1992). There are other methods of ion-paired LC analysis of milk for penicillins. The identities of the antibiotic compounds present in milk and separated by liquid chromatography are confirmed by LC-MS-MS. A simple, selective and fast multi-residue method was developed by Aguilera-Luiz *et al.* (2008) to determine 18 veterinary drugs such as quinolones, sulfonamides, macrolides, antihelminthics and tetracycline in milk by using ultra-high-pressure liquid chromatography in tandem with mass

spectrometry. The extraction procedure in this method is based on QuEChERS (quick, easy, cheap, effective, rugged and safe) and consists of a liquid extraction of the milk samples with acetonitrile. The antibiotics were detected by electro-spray ionisation in positive ion mode with multiple reaction monitoring.

There are some official microbiological assay methods available for detection of drug or antibiotic residues in milk. In affinity quantitative determination, the β-lactam antibiotics in milk can be assayed to the level of ≥ 0.01 IU penicillin G/ml or β-lactam equivalents, based on specific irreversible affinity of the antibiotics for certain enzyme sites on the cell wall of microorganisms. ^{14}C-labelled penicillin and *Bacillus stearothermophilus* are added to milk samples. The antibiotics present in the sample compete with ^{14}C-labelled penicillin for binding sites and the amount of bound ^{14}C is counted and compared with a control to determine the presence of β-lactam antibiotics (AOAC, 1995).

The microbial receptor assay for antimicrobial drugs in milk is based on the binding reaction between drug functional groups and the receptor site on added microbial cells. The ^{14}C or ^{3}H binding is measured by a scintillation counter and compared with a control milk containing no drug residues to detect anti-microbials. The greater the amount of antibiotics present in the sample, the lower the counts. However, this method does not detect metabolites, only the active drugs (AOAC, 1995). The validated levels in ng/ml are penicillin G 4.8, cephapirin 5, cloxacillin 100, chlortetracycline 2000, oxytetracycline 2000, tetracycline 2000, erythromycin 200, linomycin 400, chlindamycin 400, sulfamethazine 75, sulfamethoxazole 50, sulfasoxazole 50, streptomycin 1000, novobiocin 50 and chloramphenicol 800. Another AOAC official method for detecting β-lactam antibiotics in milk is the quantitative *Bacillus stearothermophilus* Disc Method applicable to levels ≥ 0.016 IU penicillin G/ml (AOAC, 1995).

6.3.4   Mycotoxins

Like pesticides, analysis of mycotoxins also involves three basic steps, i.e. extraction, purification and determination. The solubility of aflatoxins in organic solvents like chloroform, methanol, ethanol, acetone and acetonitrile helps in their extraction from various matrices, and their insolubility in diethyl ether and petroleum ether helps in separating them from certain interfering substances like fats and oils. Their characteristic fluorescence and absorption at long wavelengths help in their detection by UV and visible light. Mycotoxins can be detected and quantified by employing various analytical techniques such as thin-layer chromatography (TLC), GLC, high-performance TLC (HPTLC), HPLC and enzyme-linked immunosorbent assay (ELISA).

The International Dairy Federation (IDF, 1990) method of estimating aflatoxin (AFM1) in milk is briefly described as follows. Chilled milk (50 ml) is extracted with chloroform by partitioning in the presence of saturated sodium chloride solution. The chloroform layer is dried, filtered, concentrated and subjected to column chromatography using silica gel and anhydrous sodium

sulfate in equal proportions as adsorbents in chloroform. The column is subsequently eluted with toluene and glacial acetic acid (9:1 v/v), hexane and acetonitrile:diethyl ether:hexane (1:3:6 v/v) and all the fractions are discarded. Finally, the column is eluted with chloroform and acetone (4:1 v/v), and the resulting eluate contains the aflatoxins. The eluate is concentrated, dissolved in HPLC-grade acetonitrile and water (35:65 v/v) and analysed by HPLC using a C18 column and a fluorescence detector (excitation wavelength 369 nm, emission wavelength 430 nm).

For milk products the same procedure is used but with minor modification. For milk powder, 5 g of the powder is dissolved in 50 ml of warm distilled water and this solution is then extracted in a separating funnel by partitioning with chloroform in the presence of sodium dodecyl sulfate solution. Similarly, products like cheese and paneer may be shredded, mixed and extracted by shaking in a mechanical shaker with chloroform.

The confirmation of identities of aflatoxins like pesticides and other chemicals can be best done by using mass spectroscopy or LC-MS-MS. Isobe *et al.* (1988) identified 24 mycotoxins using FABMS (fast atom bombardment mass spectrometry). AFM1 in milk and milk products can also be analysed by ELISA (enzyme-linked immunosorbent assay). In this method an antibody having high affinity for AFM1 is coated on to microplate-wells or tubes. Standards or samples are added to the well and if AFM1 is present it binds to the coating antibody. Afterwards, AFM1 bound to a suitable enzyme (e.g. horse-radish peroxidase) is added, which binds to the antibody not already occupied by AFM1. After the incubation period, the reaction is stopped, the content of the well is decanted, the well is washed and the enzyme substrate is added. After enzymic hydrolysis of the substrate, a colour is produced, the absorbance of which is read in a plate reader. As the concentration of AFM1 in the sample or standard increases, the intensity of the colour is decreased (negative test). The absorbance of the sample is compared to that of the standard and the concentration in the sample is thus calculated. Bachner *et al.* (1988) used an ELISA method for detecting aflatoxins in milk. ELISA can also be used for pesticide residue analysis.

6.3.5   Nitrates and nitrites

Milk samples can be analysed for nitrates and nitrites with a limit of detection (LOD) lower than 1.0 nmol NO_3^- or NO_2^-/ml by reduction with a high-pressure cadmium column. The system allows quantitative reduction of nitrate and automatically eliminates interference from other compounds normally present in biological fluids (Green *et al.*, 1982). Viacil and Vins (1985) described a method for determination of nitrate in cow milk, milk powder, milk-based infant formula and human milk using liquid chromatography on Spheron DEAE and direct photometric detection at 205 nm after deproteinising with Carrez reagent. This method is more rapid and gives identical results to the reference method i.e. photometry after reduction of nitrate to nitrite. The LOD of the method is 0.5 mg

NO_3^-/litre of milk with 4% relative standard deviation (RSD). Another rapid and simple enzymic method was described by Priyo and Contin-Esnault (1996) for the determination of nitrate in fresh and dry milk samples after deproteinising with Carrez reagent. The interference from casein, albumin, lactose and chloride ions was greatly reduced and adequate agreement was found between results obtained by this method and those of the official reduction/photometric reference method. The LOD and LOQ (limit of quantification) were found to be 0.45 μg/ml of NO_3^- and 1 μg/ml of NO_3^- (RSD 3.4%), respectively.

6.3.6   Detergents and disinfectants

Residues of detergents and disinfectants in milk and dairy products can be detected by employing suitable techniques for the chemical type of the compounds used as detergents and disinfectants. For example, quarternary ammonium compounds may be extracted with tetraiodobismutate in CCl_4, toluene or cyclohexane and analysed by UV spectroscopy or HPLC. Other methods, using a colorimetric test, volumetric analysis or fluorescence spectroscopy, can be employed for particular types of disinfectants and detergents. The following references provide detailed methodologies for analysis of residues of detergents and disinfectants:

- Methods for chemical analysis of liquid milk and cream – Detection of detergents/disinfectants (BS 1741–11:1989), also available as British Standards Online.
- 'Detergents and disinfectants' by J Palmer (in *Monograph on Residues and Contaminants in Milk and Milk Products*), International Dairy Federation.

6.4   Regulatory aspects

The management of toxic substances involves regulating the environmental and dietary risks faced by consumers. Regulatory actions taken to eliminate or reduce environmental or occupational risks may frequently affect consumer risks since potential for exposure to the toxicants may be altered. The agreement on the application of Sanitary and Phytosanitary Measures (SPS Agreement) of the World Trade Organisation (WTO) has provided guidelines to produce safe food for international trade and recognises Codex standards on reference points related to food safety issues. The Codex Alimentarius Commission (CAC) is an intergovernmental body that sets international standards for food safety based on risk assessment in line with the SPS agreement requirements. The risk assessment considers the adverse health effects resulting from human exposure to food-borne hazards like chemical contaminants. On chemical contaminants, the work of Codex is supported by the joint FAO/WHO Expert Committee on Food Additives (JECFA), the joint FAO/WHO Meeting on Pesticide Residues (JMPR) and other experts who provide advice based on risk assessment. The JECFA was, in fact, formed after a resolution taken in 1953 at the World Health

Assembly expressing concern about the increasing use of various chemical substances in food. Codex has set standards for several chemical contaminants in many foods.

In the USA there are two nodal agencies: US-EPA (United States Environment Protection Agency) and US-FDA (United States Food and Drug Administration). The former is a standard-setting body entrusted with registration and licensing the use of pesticides, establishing the ADI (also known as the Chronic Reference Dose, CR_fD) and setting maximum residue limits (MRL) or tolerances. The US-FDA is the enforcing agency to oversee compliance with standards for domestic and imported foods. The European Union (EU) process is similar to that of the USA. The EU sets the MRL for pesticides, metals and other contaminants in food that are legally enforceable, and non-compliance leads to legal proceedings against the supplier. In other countries, including Australia, Japan and Canada, the MRL is set and food products are monitored regularly to ensure that the legal limit is not exceeded. From May 2006, the Japan Ministry of Health, Labor and Welfare has introduced a Positive List System for agricultural chemical residues in food. Under this new system, distribution of food that contains agricultural chemicals, including pesticides, feed additives and veterinary drugs, above 0.01 ppm is prohibited unless a specific MRL for the relevant agricultural chemicals has been established. In India, national food safety standards are set by the Ministry of Health and Family Welfare of the Government of India under the Prevention of Food Adulteration (PFA) Act and Rules. A new act, 'The Food Safety and Standards Act', 2006, enacted by Parliament in 2006 has replaced the PFA Act. The MRLs of pesticides, heavy metals, veterinary drugs and aflatoxins set by different agencies are set out in Tables 6.1 to 6.4.

As the role of animal feed is of paramount importance in spreading contamination to food of animal origin, so FAO, WHO, Codex and OIE have also been involved in many activities related to animal feeding, including risk assessment, development of international standards, capacity building and technical assistance to members to promote food safety. In response to its members' requests, FAO has developed a series of activities to support them in ensuring food safety. The Codex task force on animal feeding was established by the 23rd session of the CAC in July 1999 to address all issues relating to animal feeding. The main objective of the task force was to develop guidelines or standards, as appropriate, for good animal feeding practices with the aim of ensuring the safety and quality of foods of animal origin.

6.5   Management of contaminants

Feed and fodder are the main sources of contamination of animals with pesticide residues and many other contaminants. Therefore, care needs to be taken at all points of production, right from the sowing of the crops, their management, harvesting, processing and storage, so that there is ultimately no contamination

Table 6.1 Maximum residue limits (MRL) of pesticides in milk

Sl. no.	Pesticide	MRL (mg/kg)			
		Codex[a,d,e,f,g]	PFA[b,c,d]	US[b]	EU
1	2,4-D	0.01	0.05 MMP	0.05	0.01
2	2,4-DB				0.01
3	Abamectin	0.005 goat milk		0.005	0.005
4	Acephate	0.02		0.1	0.02
5	Acetamiprid			0.1	0.05
6	Acetochlor				0.01
7	Acequinocyl				0.01
8	Aclonifen				0.02
9	Acrinathrin				0.05
10	Alachlor			0.02	0.01
11	Aldicarb	0.01*			0.01
12	Aldrin and dieldrin	0.006 F EMRL	0.15 MMP FB		0.006
13	Amicarbazone			0.01	
14	Amidosulfuron				0.01
15	Aminopyralid	0.02		0.03	0.02
16	Amitraz	0.01* N		0.03/0.2 FB	
17	Anilazine				0.05
18	Aramite				0.01
19	Asulam			0.05	0.1
20	Atrazine			0.02	
21	Azadirachtin				0.01
22	Azinphos-ethyl				0.01
23	Azinphos-methyl				0.01
24	Azoxystrobin			0.006	0.01
25	Barban				0.05
26	Beflubutamid				0.05
27	Benalaxyl				0.05
28	Benfuracarb				0.05
29	Benomyl		0.1 MMP FB		
30	Benoxacor			0.01	
31	Bentazon	0.05*		0.02	0.02
32	Bifenazate	0.01 milk* 0.05 milk fat		0.02	
33	Bifenox				0.05
34	Bifenthrin	0.05 cattle milk*		0.1/1 FB	0.01
35	Binapacryl				0.01
36	Bitertanol	0.05*			0.05
37	Boscalid			0.1	0.05
38	Bromide ion				0.05
39	Bromopropylate				0.05
40	Bromoxynil			0.1	0.01
41	Bromuconazol				0.05
42	Bupirimate				0.05
43	Buprofezin			0.01	0.05
44	Butralin				0.02
45	Butylate				0.05
46	Camphechlor (Toxaphene)				0.01

Table 6.1   Continued

Sl. no.	Pesticide	MRL (mg/kg)			
		Codex[a,d,e,f,g]	PFA[b,c,d]	US[b]	EU
47	Captafol				0.01
48	Captan			0.1	
49	Carbaryl	0.05		1.0	0.05
50	Carbendazim	0.05*	0.10 MMP FB		0.05
51	Carbetamide				0.05
52	Carbofuran	0.05*	0.05 MMP FB	0.1	0.1
53	Carbosulfan	0.03*			0.05
54	Carboxin			0.05	0.05
55	Carfenatrazone-ethyl			0.05	
56	Chlorantraniprole			0.01	
57	Chlorbenside				0.05
58	Chlorbenzilate				0.1
59	Chlorbufam				0.05
60	Chlordane	0.002 F EMRL	0.05 MMP FB		0.002
61	Chlordecone				0.02
62	Chorfenson				0.05
63	Chlorfenvinphos		0.2 MMP FB		0.01
64	Chloridazon				0.1
65	Chlormequat	0.5 cattle, goat and sheep milk			0.05
66	Chloroneb		0.05		
67	Chlorthal-dimethyl				0.01
68	Chlorothalonil		0.1		0.01
69	Chlorthiamid				0.02
70	Chlorsulfuron		0.1		0.01
71	Chlorotoluron				0.05
72	Chloroxuron				0.05
73	Chlorpropham	0.0005 cattle milk F*		0.3	0.2
74	Chlorpyrifos	0.02 cattle, goat and sheep milk	0.01 MMP FB	0.01/0.25 FB	0.01
75	Chlorpyriphos-methyl	0.01*		1.25 FB	0.01
76	Clethodim	0.05*		0.05	0.05
77	Clodinafop and its S-isomers				0.02
78	Clofencet		0.02		
79	Clofentezine	0.01 cattle milk*		0.01	0.05
80	Clopyralid			0.2	0.05
81	Clothianidin			0.01	0.01
82	Copper compounds				2
83	Coumaphos			0.5 FB	
84	Cyclanilide			0.04	0.01
85	Cycloxidim				0.05
86	Cyfluthrin	0.01 cattle milk		0.2	0.02
	Cyfluthrin-beta	F N		0.2/5 FB	
87	Cyhalothrin-gamma			5.0 FB	
88	Cyhalothrin-lambda			0.4/10 FB	0.05
89	Cyhexatin	0.05* N MMP			0.05 including azocyclotin

Table 6.1   Continued

Sl. no.	Pesticide	MRL (mg/kg)			
		Codex[a,d,e,f,g]	PFA[b,c,d]	US[b]	EU
90	Cymoxanil				0.05
91	Cypermethrin	0.05 F N	0.01 MMP FB	0.1/2.5 FB	0.02
92	Zeta-cypermethrin			0.1/2.5 FB	
93	Cyproconazole			0.02	0.05
94	Cyprodinil	0.0004*			0.05
95	Cyromazine	0.01		0.05	0.02
96	Daminozide				0.05
97	DDT	0.02 F EMRL	1.25 MMP FB		0.04
98	Deltamethrin	0.05 F		0.1 FB	0.05
99	Diallate				0.2
100	Diazinon	0.02 F N			0.01
101	Dichlobenil				0.05
102	Dichlorprop				0.05
103	Dichlorvos	0.02*		0.02	
104	Diclofop				0.01
105	Dicloran				0.01
106	Dicofol	0.1 F		0.75/22 FB	0.02
107	Diethofencarb				0.05
108	Difenoconazole	0.005*		0.01	0.01
109	Diflubezuron	0.02* F		0.05	0.05
110	Diflufenican				0.05
111	Dimethachlor				0.02
112	Dimethenamid-P	0.01*			
113	Dimethoate	0.05 cattle, goat and sheep milk*		0.002	
114	Dimethomorph				0.05
115	Dimethyl carbate			0.3 FB	
116	Dimetipin	0.01*			
117	Dimoxystrobin				0.01
118	Diniconazole				0.05
119	Dinocap				0.05
120	Dinoseb				0.01
121	Dinoterb				0.05
122	Dinotefuram			0.05	
123	Dioxathion				0.05
124	Diphenyl amine	0.0004 cattle milk F*		0.01	
125	Diquat dibromide	0.01*		0.02	0.05
126	Disulfoton	0.01 cattle, goat and sheep milk			0.02
127	Dithianon			0.01	
128	Dithiocarbamates	0.05*			0.05
129	Diuron				0.05
130	DNOC				0.05
131	Dodine				0.2
132	Edifenfos		0.01 MMP FB		
133	Emamectin			0.01 FB	

Table 6.1   Continued

Sl. no.	Pesticide	MRL (mg/kg)			
		Codex[a,d,e,f,g]	PFA[b,c,d]	US[b]	EU
134	Endosulfan	0.01 milk 0.1 milk fat		2 FB	0.05
135	Epoxiconazole				0.002
136	Ethalfluralin				0.01
137	EPTC				0.02
138	Ethephon	0.05 cattle, goat and sheep milk*		0.01	0.05
139	Ethion		0.5 MMP FB		
140	Ethirimol				0.05
141	Ethofumesate				0.1
142	Ethoprophos	0.01*			
143	Ethoxyquin				0.05
144	Ethylene oxide				0.02
145	Etofenprox				0.05
146	Etoxazole			0.01 FB	
147	Etridiazole				0.05
148	Famoxadone	0.03 F		0.06 FB	0.05
149	Fenamidone			0.02	
150	Fenamiphos	0.005 F			0.005
151	Fenarimol				0.02
152	Fenazaquin				0.01
153	Fenbuconazole	0.05 cattle milk*			0.05
154	Fenbutatin oxide	0.05*		0.1 FB	0.05
155	Fenhexamid	0.01 F*			0.05
156	Fenitrothion	0.01*	0.05 MMP FB		
157	Fenoxaprop-ethyl			0.02	
158	Fenoxaprop-P				0.05
159	Fenoxycarb				0.05
160	Fenpropathrin	0.1 cattle milk F		2 FB	
161	Fenpropidin				0.01
162	Fenpropimorph	0.01			0.01
163	Fenpyroximate	0.005 cattle milk F*		0.015	0.01
164	Fenthion				0.01
165	Fentin acetate				0.05
166	Fentin hydroxide			0.06	0.05
167	Fenvalerate	0.1 F	0.01 MMP FB	0.3/7 FB	0.02
168	Fipronil	0.02 cattle milk		1.5 FB	0.005
169	Florchlorfenuron				0.05
170	Fluazifop			0.05	
171	Fluazifop-*p*-butyl				0.1
172	Fluazinam				0.05
173	Flubendiamide			0.04/0.3 FB	0.01
174	Flucarbazone-sodium			0.005	
175	Flucycloxuron				0.05
176	Flucythrinate				0.05
177	Fludioxonil	0.01			0.05
178	Flufenoxuran			0.2	0.05

Table 6.1   Continued

Sl. no.	Pesticide	MRL (mg/kg)			
		Codex[a,d,e,f,g]	PFA[b,c,d]	US[b]	EU
179	Flufenzin				0.05
180	Flumethrin	0.05 cattle milk F N			
181	Flumeturan			0.02	
182	Fluoride ion				0.2
183	Fluoxastrobin			0.02/0.5 FB	0.2
184	Fluorochloridone				0.05
185	Fluquinconazole				0.03
186	Fluridone			0.05	
187	Fluroxypyr			0.3	0.05
188	Flusilazole	0.01 cattle milk*			
189	Flutolanil	0.05*		0.05	0.05
190	Flutriafol				0.01
191	Fluvalinate-tau isomer				0.05
192	Fosetyl-Al				0.1
193	Fuberidazole				0.05
194	Furathiocarb				0.05
195	Furfural				1
196	Gibberalic acid				0.1
197	Glufosinate-ammonium	0.02*		0.15	0.1
198	Glyphosate	0.05*			0.01
199	Guazatine				0.1
200	Haloxyfop including Haloxyfop-R				0.01
201	Heptachlor	0.006 F EMRL	0.15 MMP FB		0.004
202	Hexachlorobenzene				0.01
203	Hexachlorocyclohexane (γ isomer, i.e. lindane)	0.01*	0.01 milk 0.20 MP FB		0.001
204	Hexachlorocyclohexane (α isomer)		0.05		0.004
205	Hexachlorocyclohexane (β isomer)		0.02		0.003
206	Hexachlorocyclohexane (δ isomer)		0.02		
207	Hexazinone			0.2	
208	Hexythiazox			0.02	0.02
209	Hymexazol				0.05
210	Imazalil			0.02	0.02
211	Imazapic-ammonium			0.1	
212	Imazapyr			0.01	
213	Imazaquin				0.05
214	Imidacloprid	0.02*		0.1	0.05
215	Indoxacarb	0.1 milk 2.0 milk fat		0.15/4 FB	0.02
216	Ioxynil				0.01
217	Iprodione			0.5	0.05
218	Isoproturon				0.05
219	Isoxaben				0.01
220	Isoxaflutole			0.02	

Table 6.1 Continued

Sl. no.	Pesticide	MRL (mg/kg)			
		Codex[a,d,e,f,g]	PFA[b,c,d]	US[b]	EU
221	Kresoxim-methyl	0.01*			0.05
222	Lactofen				0.01
223	Lenacil				0.1
224	Linuron			0.05	
225	Lufenuron				0.02
226	Malathion			0.5 FB	0.02
227	Maleic hydrazide				0.2
228	Mandipropamid				0.02
229	MCPA			0.1	0.05
230	Mepiquat				0.05
231	Mercury compounds				0.01
232	Metaflumizone				0.02
233	Metalaxyl			0.02	0.05
234	Metaldehyde				0.05
235	Metamitron				0.05
236	Metazachlor				0.05
237	Metconazole				0.01
238	Methabenzthiazuron				0.05
239	Methacrifos				0.01
240	Methamidophos	0.02			0.01
241	Methidathion	0.001			0.02
242	Methiocarb				0.05
243	Methomyl	0.02*			0.02
244	Methoprene	0.1 F			0.05
245	Methoxychlor				0.01
246	Methoxyfenozide	0.01		0.1	0.01
247	Metolachlor			0.02	
248	Metosulam				0.01
249	Metrafenone				0.05
250	Metribuzin			0.05	0.1
251	Metsulfuron-methyl			0.05	
252	Monocrotophos		0.02 MMP		
253	Monolinuron				0.05
254	Monuron				0.05
255	Myclobutanil	0.01 cattle milk*		0.2	0.01
256	Napropamide				0.01
257	Nicosulfuron				0.05
258	Nitrofen				0.01
259	Norflurazon			0.1	
260	Novaluron	0.40 milk 7.0 milk fat		1.0/20 FB	0.01
261	Oxadiazon				0.05
262	Oxadixyl				0.01
263	Oxamyl	0.02*			
264	Oxycarboxin				0.05
265	Oxydemeton-methyl	0.01*		0.01	0.02
266	Oxyfluorfen			0.01	0.05
267	Paclobutrazol				0.02

Table 6.1   Continued

Sl. no.	Pesticide	MRL (mg/kg)			
		Codex[a,d,e,f,g]	PFA[b,c,d]	US[b]	EU
268	Parathion				0.05
269	Parathion-methyl				0.02
270	Paraquat dichloride	0.005*	0.01	0.01	
271	Penconazole	0.01 cattle milk			0.01
272	Pencycuron				0.05
273	Pendimethanil				0.05
274	Permethrin	0.10 F		0.88/3 FB	0.05
275	Phenmedipham				0.05
276	Phenothrin				0.05
277	Phenthoate		0.01 MMP FB		
278	Phorate	0.01*	0.05 MMP FB		0.02
279	Phosalone				0.01
280	Phosmate				0.05
281	Phophines and phosphides				0.01
282	Picloram			0.05	0.05
283	Picoxystrobin				0.02
284	Pinoxaden			0.02	
285	Piperonyl butoxide	0.20 cattle milk F 0.05 excluding cattle milk		0.25 FB	
286	Pirimicarb	0.01*			0.05
287	Pirimiphos-methyl	0.01	0.05 MMP FB		0.05
288	Prochloraz	0.05*			0.02
289	Procymidone				0.05
290	Profenofos	0.01*		0.01	0.05
291	Propachlor			0.02	0.05
292	Propamocarb	0.01*			0.1
293	Propanil			0.05	
294	Propaquizafop				0.05
295	Propargite	0.10 F*		0.08/2 FB	0.1
296	Propham				0.05
297	Propiconazole	0.01*		0.05	0.01
298	Propisochlor				0.01
299	Propoxur				0.05
300	Propoxycarbazone			0.03	
301	Propyzamide			0.02	0.01
302	Prosulfocarb				0.05
303	Prosulfuron			0.01	
304	Prothioconazole			0.02	0.01
305	Pymetrozine				0.01
306	Pyraclostrobin	0.03		0.1	0.01
307	Pyrasulfutole			0.01	0.01
308	Pyrazon			0.02	
309	Pyrazophos				0.02
310	Pyrethrins			0.05 FB	0.05
311	Pyridaben			0.01	0.02
312	Pyrimethanil	0.01		0.05	

Table 6.1   Continued

Sl. no.	Pesticide	MRL (mg/kg)			
		Codex[a,d,e,f,g]	PFA[b,c,d]	US[b]	EU
313	Pyriproxyfen				0.05
314	Pyroxsulum				0.01
315	Quinclorac			0.05	
316	Quinmerac				0.05
317	Quinoxyfen	0.01 milk		85 FB	0.05
		0.20 milk fat			
318	Quintozene				0.01
319	Quizalofop-ethyl			0.01/0.25 FB	0.05
320	Resmethrin				0.1
321	Rotenone				0.01
322	S-metolachlor			0.02	
323	Sethoxydim			0.5	
324	Simazine			0.03	0.05
325	Spinetoram			0.3/7.5 FB	
326	Spinosad	1 cattle milk N		7.0	0.5
		5 cattle milk fat			
327	Spirodiclofen			0.01/0.03 FB	0.004
328	Spiromesifen			0.2 FB	0.01
329	Spirotetramat			0.01	
330	Spiroxamine				0.02
331	Sulcotrione				0.05
332	Sulfur				0.5
333	Sulfosulfuron			0.02	0.05
334	Sulfuryl fluoride			2.0	
335	Tebuconazole	0.01 cattle milk*		0.1	0.05
336	Tebufenozide	0.01*		0.04	0.05
337	Tebufenpyrad				0.05
338	Tebuthiuron			0.8	
339	Tecnazene				0.05
340	Teflubenzuron				0.05
341	Tefluthrin				0.05
342	Tepraloxydim			0.1	0.02
343	Terbufos	0.01*			0.01
344	Terbuthylazine				0.05
345	Tetraconazole			0.01/0.25 FB	0.05
346	Tetradifon				0.05
347	Thiabendazole	0.2 cattle milk			
348	Thiacloprid	0.05			0.03
349	Thiametoxam			0.02	0.02
350	Thidiazuron			0.05	
351	Thiencarbazone-methyl			0.02	
352	Thiobencarb			0.05	0.01
353	Thiophanate-methyl			0.15	0.05
354	Tolcoflos-methyl				0.05
355	Triadimefon	0.05*			0.1
356	Triadimenol	0.01* F		0.01	0.1
357	Triallate				0.05
358	Triasulfuron			0.02	

Table 6.1   Continued

Sl. no.	Pesticide	MRL (mg/kg)			
		Codex[a,d,e,f,g]	PFA[b,c,d]	US[b]	EU
359	Triazophos	0.01 cattle milk*			0.01
360	Tribufos			0.01	
361	Trichlorfon		0.05		0.1
362	Triclopyr			0.01	0.05
363	Tricyclazole				0.05
364	Tridemorph				0.05
365	Trifloxystrobin	0.02*		0.02	
366	Triflumizole			0.05	0.05
367	Triflumuron				0.01
368	Trifluralin				0.01
369	Triforine				0.05
370	Trinexapack				0.05
371	Tritosulfuron				0.01
372	Vinclozolin	0.05 cattle milk*			0.05

* At or about the limit of detection.
[a] F = residues fat soluble.
[b] FB = on fat basis.
[c] MP = milk products.
[d] MMP = milk and milk products.
[e] N = MRL accommodates external animal treatment.
[f] MRL = maximum residue limit.
[g] EMRL = extraneous maximum residue limit.

of pesticides in the fodder or feed to be fed to the animals. Comprehensive action is required from all stakeholders to keep the contamination at minimum possible levels. The government agencies should be proactive in taking various measures like proper treatment of municipal sewage, sludge and irrigation water, control of pesticide markets, sale and use of pesticides, veterinary drugs, etc. The use of good agricultural practices (GAP), good manufacturing practices (GMP) and good hygienic practices (GHP) should be invoked in all possible areas. A few points in this regard may be mentioned here.

- Seed/planting material, soil and irrigation water used for growing crops, which are supposed to be offered to animals either as main crop (fodder) or as their by-product (feed), should be free from any contamination.
- Synthetic pesticides should be used only when absolutely necessary. Integrated pest management (IPM), integrated nutrient management (INM) and organic agriculture should be introduced as much as possible, keeping in view that there is no decrease in productivity or total yield.
- Proper pesticides should be used under the proper conditions as per the manufacturer's recommendations.
- Pesticides should be applied at the exact dose.

Table 6.2   Maximum residue limits (MRL) of toxic metals in milk

Sl. no.	Metal	MRL (mg/kg)		
		Codex	PFA	EU
1	Arsenic		0.1 for milk, 1.1 for milk products, 0.05 for infant milk substitutes and infant foods, 0.5 for ice cream	
2	Cadmium		1.5 including milk products, 0.1 for infant milk substitutes and infant foods	0.5
3	Copper		30 including milk products, 15 but not less than 2.8 for infant milk substitutes and infant foods	
4	Lead	0.02	2.5 including milk products, 0.2 for infant milk substitutes and infant foods	0.02
5	Mercury		1.0 including milk products	
6	Methyl-mercury		0.25 including milk products	
7	Tin	150 for canned milk beverages, 250 for canned milk products other than beverages	250 including milk products, 5.0 for infant milk substitutes and infant foods	
8	Zinc		50 including milk products, 50 but not less than 25 for infant milk substitutes and infant foods	

- Farmers/users should not be guided by dealers/retailers in choosing chemicals.
- Application equipment should be in perfect working order and there should be minimum possible drift.
- Application should be avoided immediately before harvest.
- A safe waiting period or pre-harvest interval (the prescribed minimum time gap between the last application of a pesticide to a crop/commodity/animal and harvesting or grazing/feeding the livestock or milking/slaughtering the animal for human consumption) should be strictly maintained.
- In case of feed/fodder, the maintenance of a safe waiting period becomes all the more important and should be strictly adhered to, because the fodder is normally fed straightaway to animals and thus there is little chance for

Table 6.3   Maximum residue limits (MRL) of veterinary drugs in milk

Sl no.	Veterinary drug	MRL (μg/kg = ppb)		
		Codex	US	EU
1	Albendazole	100		
2	Amoxicillin	4	10	4
3	Ampicillin	4	10	4
4	Bacitracin	100	500	100
5	Benzylpenicillin (including procaine benzylpenicillin)	4		
6	Cefacetril	125		
7	Cefalexin	100		
8	Cefalonium	20		
9	Cefapirine	60	20	60
10	Cefazoline	50		
11	Cefoperazone	50		
12	Cefquinome	20		
13	Ceftiofur	100	100	100
14	Chlortetracycline (including oxytetracycline, tetracycline)	100		
15	Clenbuterol	0.05		
16	Clopidol		20	
17	Cloxacillin	30	10	30
18	Colistine	50		
19	CTC	100		
20	Cyfluthrin	40		
21	Cyhalothrin	30		
22	Cypermethrin and alphacypermethrin	100		
23	Danofloxacine	30		
24	Deltamethrin	30		
25	Dicloxacillin	30		
26	Dihydrostreptomycin (includes streptomycin)	200	125	200
27	Diminazene	150		
28	Doramectin	15		
29	Enrofloxacine	100		
30	Eprinomectin	20	12	20
31	Erythromycine	40		
32	Febantel (includes fenbendazole, oxfendazole)	100	60 (only fenbendazole)	60 (only fenbendazole)
33	Flumequine	50		
34	Flunixin		2	40
35	Gentamycin	100		
36	Hydrocortisone		10	
37	Imidocarb	50		
38	Isometamidium	100		
39	Ivermectin	10		
40	Kanamycin	150		
41	Linomycin	150		
42	Marbofloxacin	75		
43	Methyl prendisolone		10	

Table 6.3   Continued

Sl no.	Veterinary drug	MRL (μg/kg = ppb)		
		Codex	US	EU
44	Moxidectin		40	40
45	Nafcillin	30		
46	Neomycin	1500	1500	1500
47	Novobiocin	50	100	50
48	Oxytetracycline	100	300	100
49	Penicillins	4		
50	Pirlimycin	100	400	100
51	Rifaximine	60		
52	Spectinomycin	200		
53	Spiramycin	200		
54	Sulfadimidine	25		
55	Sulfabromomethazine sodium		10	
56	Sulfadimethoxine		10	
57	Sulphonamides (total)	100		
58	Tetracycline	100		
59	Thiabendazole	100	50	
60	Tilmicosin	50		
61	Trichlorfon (includes metrifonate)	50		
62	Tylosine	50	50	50

Table 6.4   Maximum levels of aflatoxin in milk

Toxin	MRL (μg/kg)	
	Codex	PFA
Aflatoxin M1	0.5	0.5

decontamination. Freshly treated material should never be fed to animals, particularly to those that are being milked.

- When pesticides are applied on animals by drenching their skin to control ectoparasites, milking should be avoided for a certain period depending on the type of chemical.

In spite of all efforts, if the contamination of feed and fodder with pesticides cannot be checked, there are some processes that help in decontaminating the treated materials:

- Sun-drying of the material for a few days can remove some of the residues.
- In the case of green fodders, simple washing or dipping in salt/dilute acid/ lime solution can be done to remove a certain percentage of residues. But for concentrated feed this may not be feasible. The silage-making process may also lower the concentration of residues in fresh green fodder.

If pesticides are found to be deposited in the animal body then they may be removed by different processes. These include physical methods whereby the adipose tissue, where the organochlorine pesticides are deposited, is removed surgically, or chemical methods in which, for example, pesticides can be removed by dietary administration of activated charcoal or phenobarbital or thyroprotein (Miller, 1967; Biehl and Buck, 1987; Highnight *et al.*, 1987). Some special processing techniques such as degradation of pesticides by addition of hydrogen peroxide (Cardwell *et al.*, 1966), treatment of milk fat with nitrogen (Mochalov *et al.*, 1976), vacuum deodorisation of milk fat (Ledford *et al.*, 1968), use of surface-active agents in butter oil (Liska, 1968), steam deodorisation of milk fat (Kroger, 1968), molecular distillation of anhydrous milk fat (Bills and Sloan, 1967) and use of ion exchange resin (Korolev and Bikinyaeva, 1991) have also been used.

Heavy metal pollution can be checked by following GAP and GMP. Normally very low concentrations of these metals are secreted into milk because the carryover rate of metals from forage to milk is approximately 1:500 (Bluthgen *et al.*, 1997) and mammary glands act as a biological filter against flow of heavy metals to the milk. So, if feed and fodder offered to animals are not greatly contaminated, the animals are significantly less likely to be exposed to high concentrations of heavy metals. Special care should also be taken to ensure that equipment and packaging utensils do not leach heavy metals and hence contaminate milk.

For radionuclides, various supplements including EDTA, ion exchange resins, clays, vermiculate and alginates have been fed to animals to selectively reduce the availability of ingested radionuclides (Paakkola and Wiechen, 1990). Milk is rapidly freed from all the short-lived radionuclides when refrigerated, frozen, condensed or dried (Wilson *et al.*, 1988). Cation and anion exchange resins are used for the removal of Sr, Cs and I. Electrodialysis (Macasek *et al.*, 1994), membrane osmosis and ultrafiltration (Patel and Prasad, 1992) are some other potential techniques for removal of radionuclides.

Mycotoxins are not produced in the milk but are carried over from contaminated feed. So, to manage the mycotoxin contamination in milk, every effort should be made to control fungal infection and thereby toxin production in feed at every step of their production and storage. Feed materials kept under storage for a long time and silages are more susceptible to fungal infection and toxin production, particularly by aflatoxins. Contaminated feed should never be offered to animals. Regular monitoring of feed is thus required before feeding the livestock.

In most countries, the withholding times for veterinary drugs and antibiotics are prescribed. The best preventive measure to avoid residues of these substances in milk is to strictly adhere to the respective withholding periods. Milk from treated animals is, therefore, required to be excluded from the milk supply for a specific time period to ensure that antibiotic residues no longer remain in the milk. However, the regulatory authorities and government agencies should also closely monitor the drugs and antibiotics that are administered to dairy animals to ensure that they are of the recommended type,

and are administered correctly, at the appropriate dose and time and under the supervision of veterinarians. Some processing techniques play an important role in removing drug residues from milk. The refrigeration of milk results in disappearance of the antibiotic penicillin, provided penicillinase-producing organisms are active. Some of the antibiotics lose their activity at higher temperatures encountered during boiling, pasteurisation and sterilisation (Ramakrishna *et al.*, 1985; Moats, 1988). Activated charcoal or resin and ultrafiltration using polysulfome membranes can be used to make milk free from antibiotic residues (Geyer, 1994).

Nitrate contamination can be managed by following GAP and monitoring nitrate level in forages and water offered to animals. Proper cleaning and rinsing of the udder and utensils can safeguard the milk and milk products from contamination with detergent and disinfectant residues.

6.6   Conclusions

Milk is the absolutely perfect beverage for children and teens. So, the nutritional quality and utmost safety of milk and milk products are very significant from the viewpoint of consumers as well as producers and marketing agencies. Moreover, trade liberalisation and globalisation pose a challenge in terms of ensuring quality and safety of milk products both for domestic consumption as well as for export. The health threat due to consumption of food contaminated with toxic chemicals or biological substances clearly demonstrates why stringent control measures at all stages of food production right from primary production to storage, packaging, transportation, distribution and consumption is so vital.

The very recent occurrence of melamine contamination in Chinese milk powder, its effect on babies and subsequent international repercussions is a glaring example of the sensitivity of the issue of food safety. The WHO has referred to the incident as one of the largest food safety events it has had to deal with in recent times. Melamine (1,3,5-triazine-2,4,6-triamine) is a non-protein nitrogen-rich organic heterocyclic compound primarily used in the synthesis of melamine–formaldehyde resin for the manufacture of plastics, laminates, commercial filters, glues, adhesives and moulding compounds. Although it has no nutritional value, because of its high nitrogen content melamine is added to watered-down milk to cover up the protein deficiency. Sometimes animal feeds are also tainted with melamine. Apart from baby milk powder melamine has been found in liquid milk, frozen yoghurt dessert and many non-dairy products. Other than by deliberate addition, another source of contamination may be the melamine-derivative pesticide cyromazine, which is absorbed into plants as melamine. Melamine is known to cause stones in the kidneys leading to renal problems and kidney failure in humans and animals. The use of scrap melamine (mother liquor of melamine which is impure) is even more dangerous as it contains impurities like cyanuric acid which forms more insoluble crystals than melamine alone. Concentrations of melamine in tainted milk powder

samples have been found in the range of 0.09 mg/kg to as high as 2563 mg/kg (http://en.wikipedia.org/wki/2008_Chinese_milk_scandal). According to the US-FDA, melamine below 2.5 mg/kg is not of much concern, though they are currently unable to establish any level of melamine and melamine-related compounds in infant formula that does not raise any public health concern. The EU has set 0.5 mg/kg as the safe limit of melamine.

For production of safe food for human consumption three main actions are required: setting standards for food hazards, preventive measures along the food chain, and corrective actions against unsafe food. In order to produce clean and green milk, attention should be paid from the very beginning to all the related aspects such as safe feed and fodder production and storage, animal health and shelter management, use of clean and pure water, sanitation and hygienic conditions at the animal and milking shed, use of proper utensils, their cleaning, disinfection and post-rinsing, proper packaging and storage of milk and milk products, etc. The different food safety management systems such as hazard analysis critical control point (HACCP), ISO, GAP, GMP and GHP should be implemented. International organisations such as FAO/WHO along with many countries have set MRLs for different types of contaminants, but there is a lack of harmony among the agencies. There should be harmonisation in MRLs among the different agencies including Codex, which would have to be exercised with caution, keeping in view the best interest of consumers.

6.7   Sources of further information and advice

WHO Global Strategy for Food Safety: Safer Food for Better Health (Food Safety Issues), World Health Organization, Geneva, 2002.

Monograph on Residues and Contaminants in Milk and Milk Products, International Dairy Federation, Brussels, 1997.

The international maximum residue limit database, http://www.mrldatabase.com/

Codex Alimentarius Commission (2007), List of Food Additives, http://en.wikipedia.org/wiki/List_of_food_additives_Codex_Alimentarius

Heavy Metal Contamination (http://envfor.nic.in/cpcb/hpcreport/contents.htm) (http://www.lentech.com/heavy-metals.htm)

Food Safety and Quality (http://www.fao.org/ag/agn/agns)

International Trade and Food Safety, *Economic Theory and Case Studies* (Jean C Buzby, ed.), Agricultural Economic Report No. 828, USDA, 2003.

Fermentation and Food Safety (M R Adams and M J R Nout, eds), Aspen Publishers, Gaithersburg, MD, 2001.

Mycotoxins in Dairy Products (H P Van Egmond), Elsevier Applied Science, London, 1989.

British Standards Online – Food Technology (Module 37) – Milk and Milk Products General, http://products.ihs.com/bs-seo/gbm37_17.htm

Standard Methods for the Examination of Dairy Products, 17th edition (H Michael Wehr and J F Frank, eds), Technology and Engineering, 2004.

http://en.wikipedia.org/wki/2008_Chinese_milk_scandal

http://www.who.int/foodsafety/fs_management/infosan_events/en/index.html

6.8   References

AGNIHOTRI N P (1999), *Pesticide Safety Evaluation and Monitoring*, All India Coordinated Research Project on Pesticide Residues, Indian Agricultural Research Institute, New Delhi, pp. 104–107.

AGUILERA-LUIZ M M, MARTINEZ-VIDAL J L, GONZALEZ-ROMERO R and FRENICH A G (2008), 'Multiresidue determination of veterinary drugs in milk by ultra high pressure liquid chromatography – tandem mass spectrometry', *J Chromatography A*, 1295, 10–16.

ANASTASIO A, CAGGIANO R, MACCHIATO M, PAOLO C, RAGOSTA M, PAINO S and CORTESI M L (2006), 'Heavy metal concentrations in dairy products from sheep milk collected in two regions of Southern Italy', *Acta Vet Scand*, 47, 69–74.

AOAC (1990), *Official Methods of Analysis of AOAC International*, 15th edn, pp. 237–273.

AOAC (1995), *Official Methods of Analysis of AOAC International*, 16th edn, II, pp. 38–43.

BACHNER U, MARTLBAUER E and TERPLAN G (1988), 'Detecting aflatoxin M1 in milk from selected parts of Bavaria by using an ELISA', *29. Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene Dreilandertagung*, 13–16 September 1988, Garmisch-Partenkirchen, Germany, pp. 291–294.

BALARAMAN N and GUPTA H K (1990), 'Occurrence of aflatoxin in the livestock feeds of Sikkim', *Ind J Anim Nutr*, 7, 143–146.

BATTU R S, SINGH B and KALRA R L (1996), 'Seasonal variation in residues of DDT and HCH in dairy milk in Punjab, India', *Pestic Res J*, 8, 32–37.

BATTU R S, SINGH B and KANG B K (2004), 'Contamination of liquid milk and butter with pesticide residues in Ludhiana', *Ecotoxicol Environ Saf,* 59, 324–331.

BIEHL M L and BUCK W D (1987), *J Food Prot*, 50, 1058.

BILLS D D and SLOAN J L (1967), *J Agric Food Chem*, 15, 676.

BLUTHGEN A, BURT R and HEESCHEN W H (1997), 'Heavy metals and other trace elements', in: *Monograph on Residues and Contaminants in Milk and Milk Products*, International Dairy Federation, Brussels, pp. 65–73.

BORKHATRIYA V N, SHARMA R S, CHAUDHURY P L and PATEL S M (2001), in: *International Conference on Pesticides, Environment and Food Security*, Society of Pesticide Science India, IARI, New Delhi, 19–23 November 2001.

BUCK W B (1970), *J Am Vet Med Assoc*, 156, 1434.

BUCK W B (1975), *J Am Vet Med Assoc*, 166, 222.

CARDWELL J T, MANDHARE K S and SMITH D M (1966), *J Dairy Sci*, 49, 444.

CARLSSON A and BJORCK L (1991), 'Charm rest–II for confirmation of inhibitory substances detected by differential microbial assay in herd milk', *J Food Prot*, 54, 32–36.

DIKSHITH T S S, KUMAR S N, TANDON G S, RAIZADA R B and RAY P K (1989a). 'Pesticide residues in edible oils and oilseeds', *Bull Environ Contam Toxicol*, 42, 50–56.

DIKSHITH T S S, KUMAR S N, RAIZADA R B and SRIVASTAVA M K (1989b), 'Organochlorine insecticide residues in cattle feed', *Bull Environ Contam Toxicol*, 43, 691–696.

EIDELMAN M (1962), 'Determination of micro-quantities of chlorinated organic pesticide residues in butter', *J Assoc Off Anal Chem*, 45, 672–679.

FLETOURIS D J, PSOMAS J E and MANTIS A J (1992), 'Determination of some monobasic penicillin in milk by ion pair liquid chromatography', *J Agric Food Chem*, 40, 617–621.

GARRIDO-FRENICH A, MARTNEZ-VIDAL V L, MORENO-FRIAS M, OLEA-SERRANO F and OLEA N (2000), 'Quantitative determination of endocrine disrupting polychlorinated biphenyls and organochlorinated pesticides in human serum with electron-capture detection and tandem mass sectrometry', *J Mass Spect*, 35, 967–975.

GEYER J E (1994), US Patent 5310565, cited from *Dairy Sci* Abstract 1994, 56, 5342.

GOWDA N K S, MALATHI V and SUGANTHI R U (2003), 'Screening for aflatoxin and effect of moisture, duration of storage and form of feed on fungal growth and toxin production in livestock feeds', *Anim Nutr Feed Tech*, 3, 45–51.

GREEN L C, WAGNER D A, GLOGOWSKI J, SKIPPER P L, WISHNOK J S and TANNEHBAUN S R (1982), 'Analysis of nitrate, nitrite and [^{15}N] nitrate in biological fluids', *Anal Biochem*, 126, 131–138.

GRIFFITH K R and CRAUN J C (1974), 'Gel permeation chromatographic system: an evaluation', *J Assoc Off Anal Chem*, 57, 168–172.

HEATH A B and BLACK R R (1979), 'Investigation and simplification of the sweep codistillation clean up of pesticide residues in animal fats', *J Assoc Off Anal Chem*, 62, 757–763.

HEATH A B and BLACK R R (1980), 'Improvements to assisted distillation clean up of pesticide residues in animal fats', *J Assoc Off Anal Chem*, 63, 529–531.

HIGHNIGHT S D, DANIELS L B, KELLOG D W and NAJAD H H (1987), *J Dairy Sci* (Suppl 1), 70, 252.

IDF (1990), 'Milk and dried milk: Determination of aflatoxin M1 content', IDF Standards, IDF-111A, International Dairy Federation, Brussels.

ISOBE M, UYAKUL D and GOTO T (1988), 'Mycotoxin analysis by FAB-MS/MS spectrometry', *Proc Japanese Assoc Mycotox* , Supplement 1, 49–50.

JOHNSON L D, WALTZ R H, USSAY J P and KAISER F E (1976), *J Assoc Off Anal Chem*, 59, 174–187.

KANG B K, SINGH B, CHAHAL K K and BATTU R S (2002), 'Contamination of feed concentrates and green fodder with pesticide residues', *Pestic Res J*, 4, 308–312.

KAPHALIA B S and SETH T D (1982), 'Organochlorine pesticides contamination in some species of fodder grasses', *Environ Pollut* Series B 3, 231–237.

KAPOOR S K, CHAWLA R P and KALRA R L (1981), 'Simplified method for estimation of DDT and hexachlorocyclohexane residues in milk', *J Assoc Off Anal Chem*, 64, 14–15.

KOROLEV B A and BIKINYAEVA R KH (1991), *Sibirskii Vestnik Sel'skokhozyaistvennoi Nauki*, 2, 72 (*Dairy Sci* Abstract 1994, 56, 4450).

KROGER M (1968), *J Dairy Sci*, 51, 196.

LEDFORD R A, CHERN J H and SHIPE W F (1968), *J Dairy Sci*, 51, 219.

LISKA B J (1968), *J Anim Sci*, 27, 827.

LUKE M A and DOOSE G M (1984), 'A rapid analysis for pesticides in milk and oilseeds', *Bull Environ Contam Toxicol*, 32, 651–656.

MACASEK F, GERHART P and MALOVIKOVA A (1994), *J Radioactivity Nuclear Chem*, 18, 99.

MCCULLY K A and MCKINLEY W P (1964), 'Determination of chlorinated pesticide residues in fat by electron capture gas chromatography', *J Assoc Off Anal Chem*, 47, 652–659.

MEETSCHEN U and PETZ M (1991), 'Gaschromatographische Analysenmethode für Rückstände von sieben Penicillinen in Lebensmitteln tierischen Ursprungs', *Z Lebensm Unters Forsch*, 193, 337–343.

MILLER D D (1967), *J Dairy Sci*, 50, 1444.

MILLS P A, BONG B A, KAMPS L R and BURKE J A (1972), 'Elution solvent system for florisil column clean up in organochlorine pesticide residue analysis', *J Assoc Off Anal*

Chem, 55, 39–43.

MISHRA R S and SINGH R S (1978), 'Aflatoxin contamination of grains in flooded area of Mathura, Uttar Pradesh', *Curr Sci*, 47, 396–99.

MOATS W A (1988), *J Food Prot*, 51, 491.

MOATS W A and MALISCH R (1992), 'Determination of cloxacillin and penicillin in milk using an automated liquid chromatography clean', *J Assoc Off Anal Chem Int*, 75, 257–260.

MOCHALOV V I, MOLOCHNIKOV V V and CHUDAKOVA L N (1976), *Trudy-Vsesoyuznyi-Nauchno-issledovatel'skii-Institut-Molochnoi-Promyshlennosti*, 43, 59 (*Dairy Sci* Abstract, 1977, 39, 4681).

MUAN B and SKAREE J U (1986), 'Gas chromatographic determination and mass spectrometric confirmation of malathion in milk and blood', *J Agric Food Chem*, 34, 87–88.

MUÑOZ E and PALMERO S (2004), 'Determination of heavy metals in milk by potentiometric stripping analysis using a home-made flow cell', *Food Control*, 15, 635–641.

NAG S K and RAIKWAR M K (2006), Final Report of Lal Bahadur Shastri Young Scientist Scheme: 'Monitoring of pesticide residues in animal feed, fodder and milk with special reference to Bundelkhand region', submitted to ICAR, New Delhi.

PAAKKOLA O and WIECHEN A (1990), *Int Dairy Fed Bull*, 247, 3.

PARDIO V T, WALISZEWSKI K N, LANDIN L A and BAUTISTA R G (2003), 'Organochlorine pesticide residue in cow's milk from a tropical region of Mexico', *Food Addit Contam*, 20, 259–269.

PATEL P A and PRASAD S R (1992), *Indian Dairyman*, 44, 572.

PATEL P M, NETKE S P, GUPTA B S and DABADGHAO A K (1981), 'Note on the survey of consumer milk supplied to Jabalpur city for the incidence of aflatoxin M_1 and M_2', *Ind J Anim Sci*, 51, 906.

PIERSON D A, HOFFMAN J S, NORD P J, GEBHART J E and FRANK C W (1982), 'Distribution of chlorinated pesticides in animal feed components and finished feeds', *J Agric Food Chem*, 30: 187–189.

POLAN C F, NAYES J R and CAMPBELL T C (1974), *J Agric Food Chem*, 22, 635.

PRASAD K S N, CHOPRA R C, CHHABRA A, DUDHE A, MURTHY T N and PRASAD T (1997), 'Survey of common feedstuffs for aflatoxin B1 contamination in and around Karnal district in Haryana', *Ind J Anim Nutr*, 14, 269–271.

PRIYO B V and CONTIN-ESNAULT D (1996), 'A survey of nitrate contents in Indonesian milk by enzymatic analysis', *Food Addit Contam*, 13, 77–87.

RAMAKRISHNA Y, SINGH R S and ANAND S K (1985), *Egypt J Dairy Sci*, 13, 85.

RAMPAL B, VARMA K and SRIVASTAVA D D (1979), 'Aflatoxin in groundnut oil, groundnut cake and hydrogenated oil in Hapur (UP) India market', *J Food Sci Tech* 16, 169–170.

ROGERS W M (1972), 'Use of a solid support for the extraction of chlorinated pesticides from large quantities of fats and oils', *J Assoc Off Anal Chem*, 55, 1053–1057.

SALAS J H, GONZALEZ M M, NOA M, PEREZ N A, DIAZ G, GUTIERREZ R, ZAZUETA H and OSUNA I (2003), 'Organophosphate pesticide residues in Mexican commercial pasteurized milk', *J Agric Food Chem*, 51, 4468–4471.

SHARMA V, WADHWA B K and STAN H J (2005), 'Multiresidue analysis of pesticide in animal feed concentrate', *Bull Environ Contam Toxicol*, 72, 242–245.

SHASTRY M S (1983), 'Monitoring of pesticide residues in animal feeds and animal products', *Pesticides*, 10, 36–38.

SINGH P P, BATTU R S and KALRA R L (1988), 'Insecticide residues in wheat grain and straw

arising from their storage in premises treated with BHC and DDT under malaria control programme', *Bull Environ Contam Toxicol*, 40, 696–702.

SINGH R, KUMAR B, MADAN V K, KUMAR R and KATHPAL T S (1997), 'Monitoring of HCH residues in animal feeds', *Ind J Anim Sci*, 67, 250–252.

SPECHT W and TILKES M (1980), *Fresenius Z Anal Chem*, 301, 300–307.

STIJVE T (1983), 'Miniaturised methods for monitoring organochlorine pesticide residues in milk', in: Miyamoto J and Kearney P C (eds), *Pesticide Chemistry: Human Welfare and the Environment*, Pergamon Press, Elmesford, NY, 4, 95–100.

STIMAC R M (1979), 'Rapid florisil clean up method for analysis of chlorinated pesticide residues', *J Assoc Off Anal Chem*, 62, 85–88.

SUZUKI T, ISHIKAWA K, SATO N and SAKAI K I (1979), 'Determination of chlorinated pesticide residues in foods. III. Simultaneous analysis of chlorinated pesticide and phthalate ester residues by using AgNO$_3$ coated florisil column chromatography for clean up of various samples', *J Assoc Off Anal Chem*, 62, 689–694.

TELLING G M and SISONS D J (1977), *J Chromatography*, 137, 405–427.

TESSARI J D and SAVAGE E P (1980), 'Gas–liquid chromatographic determination of organochlorine pesticides and polychlorinated biphenyls in human milk', *J Assoc Off Anal Chem*, 63, 736–741.

TOYODA M, ADACHI K, IDA T, NODA K and MINAGAWA N (1990), 'Simple analytical method for organophosphate pesticide residues in milk', *J Assoc Off Anal Chem*, 73, 770–772.

ÜMIT A Y and KARAYÜNLÜ S (2008), 'Modification in direct analysis method: metal levels in raw milk at the region of Izmit', *Int J Food Sci Tech*, 43, 326–329.

UNNIKRISHNAN V, SURENDRA NATH B, GAYATHRI V, SAMPATH K T and RAMA MURTHY M K (1998), 'Organochlorine pesticide residues content in feed and feedstuff', *Ind J Dairy Biosci*, 9, 59–64.

VALIUKENAITE R, JARMALAITE I, STANKEVICIENE M and STANKEVICIUS H (2005), 'Review of heavy metals in cow's milk', *Veterinarija Ir Zootechnica*, 29, 51.

VAN EGMOND H P (1989), 'Aflatoxin M$_1$: Occurrence, toxicity, regulation', in Van Egmond H P (ed.), *Mycotoxins in Dairy Products*, Elsevier Applied Science, London, pp. 11–55.

VAN HOUWELING C D, BIXLER W B and MCDOWELL J R (1977), *J Am Vet Med Assoc*, 171, 1153.

VEIEROV D and AHARONSON N (1978), 'Simplified fat extraction with sulfuric acid as clean up procedure for residue determination of chlorinated hydrocarbons in butter', *J Assoc Off Anal Chem*, 61, 253–260.

VEIEROV D and AHARONSON N (1980), 'Improved clean up of large lipid samples for electron capture gas chromatographic quantitation and gas chromatographic–mass spectrometric confirmation of organochlorine residues', *J Assoc Off Anal Chem*, 63, 202–207.

VIACIL F and VINS I (1985), 'Determination of nitrates in milk by ion chromatography', *Food*, 29, 467–472.

WALISZEWSKI S M and SZYMCZYNSKI G A (1982), 'Simple low cost method for determination of selected chlorinated pesticides in fat samples', *J Assoc Off Anal Chem*, 65, 677–679.

WILSON L G, BOTTOMLEY R C, SUTTON P K and SISK C M (1988), *J Soc Dairy Technol*, 41, 10.

WONG S K and LEE W O (1997), 'Survey of organochlorine pesticide residues in milk in Hong-Kong (1993–1995)', *J Assoc Off Anal Chem Int*, 80, 1332–1335.

7

Good hygienic practice in milk production and processing

M. C. te Giffel and M. H. J. Wells-Bennik, NIZO food research, The Netherlands

Abstract: This chapter describes the principal microbial hazards concerning milk and milk products and the main transmission routes of microbial contaminants to raw milk. The importance of good hygienic practice measures at the farm level, during processing and at the consumer level are discussed.

Key words: microbial contaminants, hygiene, farm, milking installation, processing.

7.1   Introduction

Milk and dairy products are highly nutritious media, in which micro-organisms can multiply and cause spoilage. The levels and types of micro-organisms in milk and dairy products depend on the initial levels present in raw milk, the microbial quality of other raw materials, the conditions under which the products are produced and the temperature and duration of storage. The most common spoilage micro-organisms of milk and dairy products are Gram-negative rod-shaped bacteria (e.g. *Pseudomonas* spp., coliforms), Gram-positive spore-forming bacteria (e.g. *Bacillus* spp., *Clostridium* spp.), lactic acid producing bacteria (e.g. *Streptococcus* spp.) and yeasts and moulds.

Milk and milk products have in certain instances been associated with foodborne illness. In the USA milk and dairy products were involved in approximately 4% of the foodborne bacterial disease outbreaks with known

vehicles in 2007, i.e. 250 reported cases. In the EU, investigations of foodborne diseases in 2007 showed that dairy products were involved in about 3% of reported cases with known etiological agents. Disease is mainly due to consumption of unpasteurized milk containing pathogenic micro-organisms (e.g. *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, and importantly *Campylobacter*) or products that have been contaminated after production or have undergone temperature abuse (e.g. calicivirus including norovirus, *Staphylococcus aureus*).

In this chapter, the principal microbial hazards concerning milk and milk products, focused on heat-treated liquid milk products, will be described. The importance of good hygienic practice measures and dairy product safety systems at farm, processing plant and consumer level will be discussed. Finally, some future trends in dairy processing are presented.

7.2   The principal hazards

7.2.1   Raw milk

Raw milk, as secreted by healthy cows, is free of micro-organisms. However, micro-organisms associated with the teat move up the teat canal and into the interior of the udder. Most of the bacteria present in raw milk are contaminants of the outside and gain entrance into the milk from various sources including soil, bedding, manure, feed and milking equipment. Therefore, raw milk contains levels of a few to several thousands of bacteria per ml. The microbial quality and the composition of the microflora of raw milk vary with seasons. Improvement of handling and processing of milk such as developments in closed milking systems, use of bulk tanks to store and transport raw milk and changes in refrigeration systems have resulted in shifts in the microflora from predominantly Gram-positive, acid-producing bacteria to Gram-negative, psychrotrophic micro-organisms, mainly *Pseudomonas* species. These microorganisms grow rapidly at refrigeration temperatures and produce heat-resistant extracellular proteolytic and lipolytic enzymes that survive heat processing. Enzyme activity during storage will result in defects in flavour, texture and stability in milk and dairy products.

A variety of pathogenic bacteria have been isolated from raw milk including *Mycobacterium* spp., *Salmonella*, *Listeria monocytogenes*, *Bacillus cereus*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Escherichia coli* and *Staphylococcus aureus*. Depending on the country of origin, species, climate and sanitary conditions, raw milk can contain one or more of the pathogens listed.

7.2.2   Pasteurized milk

The health rules in the EU for the production and placing on the market of raw milk, heat-treated milk and milk-based products are described in Council

Directive 92/46/EEC. Pasteurization is applied to inactivate heat-sensitive spoilage and pathogenic bacteria present in the raw milk. The minimum requirements to destroy potential pathogenic micro-organisms are pasteurization for 15 s at 71.7°C or for 30 min at 62.7°C. In Fig. 7.1, the inactivation of vegetative (pathogenic) micro-organisms (i.e. *Listeria monocytogenes* and *Salmonella*) and bacterial spores (*Clostridium tyrobutyricum* and *Geobacillus stearothermophilus*) by various heat treatments is shown. The figures demonstrate that the spores are much more resistant to heat than the vegetative micro-organisms. As can be observed, thermization and pasteurization are not sufficient to inactivate bacterial spores.

Further processing steps (pH reduction through the addition of starter, drying, addition of salt and cooling) are designed to limit the growth of the thermoduric bacteria that survive the heat treatment. Spoilage of pasteurized milk products is caused by growth and enzyme production by psychrotrophs before pasteurization and/or by activity of thermoresistant enzymes. The effectiveness of cleaning and sanitizing procedures greatly influences the level of contamination and the types of micro-organisms introduced via equipment.

Trends in the dairy industry such as the extended refrigerated storage of raw milk prior to processing, the application of higher pasteurization temperatures and the more effective control of post-pasteurization contamination have enhanced the importance of thermoduric psychrotrophs. Spore-forming bacteria,

Fig. 7.1   Inactivation of micro-organisms in raw milk by various heat treatments applied in the processing of milk and dairy products.

predominantly *Bacillus* species, limit the shelf-life of pasteurized milk and milk products. Especially *B. cereus* is associated with defects such as off-flavours, sweet curdling and bitty cream caused by proteinase, lipase and phospholipases produced by the bacteria. Several studies have shown that *B. cereus* was present in pasteurized milk after storage.

   In properly processed dairy products, most pathogens are not considered a problem, since pasteurization is effective in destroying these organisms. However, several cases of foodborne illness have been reported for, e.g., *Salmonella*, *Listeria*, *E. coli* and *Yersinia*, due to post-pasteurization contamination. Production of heat-stable enterotoxins by *Staph. aureus* in raw milk may also cause disease via various dairy products including pasteurized milk, cheese, ice cream, butter and non-fat dry milk.

7.2.3   UHT-milk

Sterilization is intended to inactivate all the micro-organisms present, both vegetative forms and spores, or at least make them incapable of growth in the product, so that a long keeping quality is obtained without refrigerated storage.

   The Milk Hygiene Directive 92/46/EEC demands that the minimum heating temperature for the manufacture of UHT-milk should be 135°C with a minimum holding time of 1 s. Typical time–temperature combinations applied in the dairy industry are holding times of the order of a few seconds at temperatures ranging from 135 to 150°C.

   There are many different types of UHT-sterilizing equipment. The principles of operation and construction of the main types of equipment are summarized in Fig. 7.2. Microbial spoilage of UHT-milk may occur by outgrowth of spores, surviving the heat processing, or by post-process contamination after heat processing (e.g. via packaging material or cooling water) or a failure in the thermal process. Typical spoilage organisms include thermoduric and spore-forming bacteria such as *Bacillus* species, *Geobacillus* species, *Streptococcus* and *Micrococcus* and occasionally some Gram-negative bacteria.

   During the last decades, highly heat-resistant mesophilic spores (HRS) have been reported in UHT-sterilized dairy products, causing non-sterility and

Fig. 7.2   Types of UHT-processing equipment.

defects, including gas production, acid coagulation, thinning, bitterness and off-odours, of, for instance, milk, chocolate milk, evaporated milk, reconstituted milk and cream. Heat-resistant sporeformers encountered in dairy products include strains of *Bacillus sporothermodurans*, *B. subtilis*, *B. circulans*, *B. coagulans* and *G. stearothermophilus*. A study in which the heat resistance of *B. sporothermodurans* isolates was assessed showed high heat resistance of this strain in the UHT-region, with D140 values ranging from 3.4 to 7.9 s, compared with *G. stearothermophilus* with a D140 value of 0.9 s. In the range 110–120°C, the spores of *B. sporothermodurans* are just as heat resistant as (or less than) those of *G. stearothermophilus*. This is shown in Fig. 7.3. *Bacillus sporothermo-durans* is not a risk to the health of consumers. However, dairies are forced to manage the problem due to legal requirements and to avoid trade restrictions. To control *B. sporothermodurans*, direct or indirect heating processes reaching F_0 values of 50 are necessary.

In addition to microbial spoilage of UHT-milk, gelation and coagulation of milk proteins and off-flavour formation may also occur as a result of heat-resistant proteolytic or lipolytic enzymes produced in the raw milk during storage. Proteolytic enzymes, naturally present in milk, probably originating from blood, are heat resistant. Studies have shown that these proteinases could survive UHT-processing.

Fig. 7.3   Thermal death time curves of *B. stearothermophilus* spores (■) and *B. sporothermodurans* J16 (○); best fit lines through experimental data.

7.3   Good hygienic practice

The various stages in the milk processing chain, from milking the cow to consumption, must be properly controlled to assure the quality and safety of milk and dairy products. Adherence to basic good agricultural practices and good manufacturing practices is one of the first steps to achieve this. Furthermore, HACCP can be applied as a tool to assess hazards and establish control systems that focus on preventive measures rather than relying mainly on end-product testing. Critical key aspects with respect to milk and dairy products are ensuring that raw materials are of good quality, and the elimination of spoilage and pathogenic bacteria from raw milk and other raw materials by heat treatment, prevention of subsequent contamination, and growth limitation of undesirable micro-organisms during storage prior to consumption.

7.3.1   Farm

Micro-organisms and spores are widespread in the natural environment, with soil, water, plants and animals serving as reservoirs. Some degree of contamination of raw milk during production is inevitable, with milking and milk storage equipment being the major sources of contamination. If milk is produced under sanitary conditions, the typical bacteria of the udder surface, mainly micrococcaceae, predominate and less than 10% of the total flora consists of psychrotrophs. Under unsanitary conditions of production, milk can contain more than 75% psychrotrophs.

Transmission of microorganisms to raw milk occurs via three main routes, namely, (1) the farm environment by contamination of the exterior of teats, (2) the interior of teats, for example in the case of (subclinical) mastitis, and (3) the milking installation.

The most common contamination sources in the farm environment are feeds, faeces, bedding material and soil. Contamination of raw milk via feed mainly occurs after passage through the alimentary tract and emission via the faeces. The impact of feed as a hazard with regard to microbial contaminants is twofold: feed can be a source of pathogens causing infection in cattle, and a source of bacterial spores. Pathogenic microbes associated with feed include *Listeria monocytogenes*, *E. coli* and *Salmonella.* These microbes are inactivated during pasteurization, but are of particular concern for raw milk products. Spore-forming bacteria that have been isolated from feeds belong to the genera *Clostridium* and *Bacillus*. *Clostridium tyrobutyricum* is a species that is well known to be associated with low-quality silage, and can cause defects in Gouda and Emmenthaler type cheeses (off-flavours and excessive gas forming). Other sporeformers in feed that can cause problems in final products are highly heat-resistant spores that survive UHT processing. Contamination of the exterior of teats and subsequent contamination of raw milk is due not only to exposure of teats to faeces, but also possibly to contact with bedding materials and soil. Again, pathogenic microbes and sporeformers constitute the main microbial hazards. Soil is generally recognized as a main source of psychrotrophic

sporeformers that survive the pasteurization process and determine the shelf-life of pasteurized milk.

Whereas milk is sterile when secreted in the alveoli of the udder in healthy cows, mastitis organisms can enter the teat canal and infect the interior of the teats. When infection progresses, these micro-organisms can be shed to the milk during the milking process. The concentration of mastitis associated micro-organisms in bulk tank milk depends on the type of organism, the infection status of the herd (clinical/sub-clinical), the stage of infection, and the fraction of the herd infected. Mastitis can be caused by environmental pathogens, or pathogens that are transferred from cow to cow with or without intermediate vectors such as teat holders. The most important contagious pathogens include *Staph. aureus*, *Streptococcus agalactiae* and *Corynebacterium bovis*. Pathogens present in the farm environment can also contaminate teats via soiling, upon which these microorganisms can enter the teat canals and subsequently cause infection. The most important pathogens implicated in these type of infections include *Streptococcus uberis*, *S. dysgalactiae* and Gram-negative bacteria such as *Escherichia coli* and *Klebsiella* spp.

Lastly, the milking installation can be a source of bacterial contamination of raw milk when bacteria adhere to surfaces or when milk residues remain in the equipment after the cleaning cycle. Growth of bacterial contaminants may occur in the installation, followed by shedding of organisms in the milk during the next milking procedure. In general, microbes that are present in the farm environment can also be found on equipment surfaces. The cleaning procedure of the equipment is important to remove residues, and the design and maintenance of the equipment is important to prevent buildup of bacterial populations in the milking installation. The occurrence of various bacterial species adhering to rubber and stainless steel in a milking installation has been reported. Gram-negative organisms predominated (96–100%), the majority being *Acinetobacter* spp., followed by *Pseudomonas* spp. and *Flavobacterium* spp.

The numbers and types of micro-organisms that develop subsequently during refrigerated storage in the bulk tank and during refrigerated transport to the factory are determined by the temperature and duration of the storage. Temperatures must be under 6°C when milk is not collected daily, and under 8°C when milk is collected daily (European Commission Regulation 852/2004). The raw milk must be transported to the dairy under such conditions that the microbiological quality of the milk is not reduced. Milk collection tankers should be designed and constructed according to the IDF Code of Practice for Design and Construction of Milk Collection Tankers (IDF Document 128). During transport, the temperature of the milk should not exceed 7°C. Insulation and refrigeration of milk tankers may be necessary under some climatic conditions. The milk tanker should be cleaned and disinfected at least daily and whenever there is a gap of four hours or greater between collections. The sufficiency of cleaning and disinfection should be checked regularly. However, even under such conditions, psychrotrophic bacteria such as *Pseudomonas* and *Listeria* species and *B. cereus/weihenstephanensis* are able to grow.

It is unlikely that all bacteria can be eliminated from the raw milk supply. Most important is to minimize contamination at the farm by control of microbial contaminants in feed, facility hygiene, cleanliness of cows (teats and udders), good animal health management to avoid mastitis, effective cleaning and disinfection procedures of the milking installation, and rapid cooling to temperatures of 4ºC or less.

7.3.2   Processing

At all stages in processing, good hygiene of the manufacturing plant is essential to ensure that the product stream is not (re)contaminated after heat treatment of raw milk (pasteurization or UHT-sterilization). Sources of post-pasteurization include equipment, packaging materials, air, aerosols, (condensed) water, lubricants, etc. Pasteurization equipment should be properly designed, installed, maintained and operated to ensure that the milk is heated to at least the specified temperature for at least the specified time.

Requirements for good hygiene design of food processing equipment, including dairy equipment, are described in various directives of the European Commission, the Hygiene of Foodstuffs' Directive and the Machinery Safety Directive (89/392/EEC). In addition, CEN/TC 153 has produced a European Standard on the hygienic requirements for food processing machinery to support 89/392/EEC.

Various organizations such as the European Hygienic Equipment Design Group (EHEDG), the International Dairy Federation (IDF), the 3-A organization and the International Standardization Organization (ISO, Technical Committee 199) have formulated and published (voluntary) principles of hygienic and aseptic design, requirements for hygienic and aseptic equipment and methods to test whether equipment fulfils these requirements. Guidance on design, construction and installation of equipment, cleaning-in-place (CIP) systems and plant is given in various IDF documents, e.g. IDF Docs 117, 123, 218 and 292. Summaries of EHEDG guideline documents are published by Elsevier in *Trends in Food Science and Technology*. 3-A sanitary standards are available for many types of equipment, from fittings to silo tanks. Documents are published in *Dairy, Food and Environmental Sanitation* (*DFES*) magazine.

To maintain the factory environment in a hygienic condition, cleaning programmes should be established. Most of the equipment used for handling milk and milk products is cleaned and disinfected by CIP systems at least daily. Start-up of closed processing lines in the dairy industry is usually done by circulating hot water in order to have additional decontamination of the equipment. Monitoring CIP systems, i.e. concentrations of the cleaning agents, temperatures, flow, pressure and circulation time, is necessary to ensure the efficiency of cleaning.

Biofilms present on the surface of milk processing equipment threaten the quality and safety of dairy products. Dead ends, corners, cracks, crevices, gaskets, valves and joints in the processing equipment are vulnerable points for

biofilm accumulation. Development of biofilms in a dairy manufacturing plant depends on the type of micro-organism, the type of product being processed, the operating conditions of the plant (temperatures, length of production runs) and the type of surface. The hygienic design of processing equipment is of great importance in avoiding biofilm formation. Biofilm control also relies on well-defined cleaning and sanitizing procedures and the effectiveness of these procedures. Bacteria within biofilms are more difficult to eliminate than free-living cells and once established can act as a source of contamination. Contamination attributed to biofilm development has been reported in general milk processing (e.g. pasteurization and milk transfer line) and the manufacture of cheese, whey and milk powder. Pathogenic micro-organisms, including *Listeria monocytogenes*, *Salmonella typhimurium* and *Yersinia enterocolitica*, will also attach to surfaces in dairy processing environments, e.g. stainless steel. Subsequently, dairy products may be contaminated.

The pasteurizer can be a source of contamination of *Bacillus* spp. In addition, the growth of bacteria, e.g. *Streptococcus thermophilus*, on the surface in the regeneration section of plate heat exchangers can contaminate milk with 100 to 10^6 bacteria per ml and/or their metabolic products (Figs 7.4 and 7.5). During the manufacture of whole milk powder, spores of thermophilic bacteria are of particular concern, as these can be formed in the preheater plate heat exchanger and in the evaporator, leading to high levels of heat-resistant spores in the final product.

Fouling must be controlled well, as not only does this affect the quality of products manufactured from this milk, but necessary extra cleaning procedures also lead to increased use of energy, a decrease in production time, and possibly corrosion, causing considerable economic loss. The filling machine is a significant source of post-pasteurization contamination. The presence of spoilage psychrotrophs (*Acinetobacter*, *Pseudomonas* and *Flavobacterium* spp.) in pasteurized milk is considered to occur after pasteurization and indicates inadequate cleaning.

Packaging material, carton-forming mandrels, filling heads and airborne micro-organisms have also been identified as major contamination sources.

Fig. 7.4   Fouling of heat exchangers can lead to shedding of microbial contaminants from the precipitation.

Fig. 7.5   Adherence of bacterial spores on equipment surfaces: (a) vegetative bacteria and spore-forming bacteria adhered to a surface; (b) spores survive cleaning and disinfection; (c) outgrowth of spores and release of vegetative cells into the bulk-phase, i.e. the food product; (d) adherence of bacteria, release of bacteria and metabolic products into the bulk-phase.

Food-grade paper and board used in the dairy industry are usually of high hygienic quality and microbial counts are well below the limits set by the FDA, 1 cfu/cm^2 or 250 cfu/g. In a study it was demonstrated that the contamination of the inner surface of cartons intended for liquid foods rarely exceeded 10 cfu per package of one litre capacity. Reusable milk bottles have been shown to be contaminated by spore-forming organisms such as *B. cereus* in concentrations of 10 to 250 per 100 ml rinsing water.

7.3.3   End-products

The microbiological quality and shelf-life of end products are determined by the microbiological quality of the raw milk, the time and temperature of pasteurization, the presence and activity of post-pasteurization contaminants, the types and activity of pasteurization-resistant micro-organisms and the storage temperature of milk after pasteurization.

The relation between storage temperature and shelf-life of pasteurized milk is well recognized. Sporeforming bacteria constitute the major spoilage flora of pasteurized milk. Low temperatures retard the growth of bacteria and conversely increase shelf-life. In Fig. 7.6 the percentage of samples containing more than five *B. cereus* per ml in household refrigerators is shown as a function of the temperature measured in the refrigerator and the time to expiry date of the milk. As expected, the level of *B. cereus* present in milk increased with storage time

Fig. 7.6   Incidence of *B. cereus* in pasteurized milk (100 ml samples) in households in relation to storage time and temperature of the refrigerator (☐ < 7ºC; ■ >7ºC).

and temperature. The temperature should be maintained at less than 4ºC in the distribution chain to reduce growth of psychrotrophs. Monitoring of the temperatures and information on temperature history can be used to identify problem areas and allow improvements to be made.

Training programmes could ensure that all people involved in processing, distribution and handling of milk and dairy products understand the principles of personal hygiene, milk spoilage and the need to keep milk cold constantly. Consumers also have to be educated as to the importance of keeping milk cold. Label information on packages may help to achieve improved quality control of milk and dairy products.

7.4   Future trends

7.4.1   Farm

In order to assure high quality standards for dairy products, it is necessary to manage the whole production chain from farm to consumer. The chains at the beginning of the primary production site, including the feed chain, are becoming more and more important. Herd management and milking processes are also critical factors in the quality of raw milk. One of the major developments in the last decades is the introduction of automatic milking systems. Since their intro-duction in 1992, the use of automatic milking systems has increased rapidly, to the point that it is now an established management system in Europe. Benefits of the systems include reduced labour demand, improved animal health and welfare

and increased milk yields. With respect to control of the bacteriological quality of the milk, important aspects of automatic milking systems are cleaning of teats and milking equipment and the (direct or indirect) cooling systems. Another development is the use of alternative feed supplies (e.g. side streams originating from biofuel production). These feeds potentially contain microbial populations that are different from those found in the more conventional feed supplies.

7.4.2   Product and process development

Consumer demands for healthier foods also influence the development of dairy products. The current generation of dairy products contains those that have been nutritionally improved by enhanced formulations of traditional dairy products. Modifications of dairy products include modifications or reductions in fat, cholesterol, sodium or calories and addition of beneficial components such as calcium.

   The dairy industry can meet the needs of consumers and expand the dairy product market by undertaking new approaches to processing and product development. Target areas that have potential are, e.g., traditional products that indulge but balance nutrition, new product concepts utilizing dairy components, food service products, processing and formulation technologies to extend shelf-life to 20–45 days, new packaging strategies, convenience, excellent sensory characteristics and safety. Innovative new technologies (e.g. high-pressure processing, separation technologies) or alternative uses of existing technologies (e.g. steam infusion, microfiltration, bactofugation) can be applied for the development of new products or new product formulations. Biotechnological and separation technologies can provide ingredients, isolated dairy components and bacterial cultures that are important for developing new dairy formulations. Many of the technologies are still capital intensive. Furthermore, safety issues relevant to new formulations and processing conditions for extended shelf-life products will continually remain a challenge for the food and dairy industries.

7.4.3   Monitoring, control and optimization of production processes

The food industry has concentrated on examination of end-products for controlling production processes. The accent shifted from analysis at the end of the process to control of the process, by the introduction of Good Manufacturing Practice (GMP) and the Hazard Analysis of Critical Control Points (HACCP) system. In HACCP systems, microbiological methods are needed for, among other things, assessing the quality of the raw materials, detecting micro-organisms in process lines and the environment, and validation and verification. A measuring system (control measure) is necessary to make sure that the critical control points (CCPs) are controlled indeed. Most ideal is a continuous registration system by means of physical and chemical analyses. Developments in the area of sensors will continue and lead to applications within the food industry within the next 10 years. Classical microbiological tests are unsuitable for

quickly obtaining current measuring data and readjusting processes. Therefore, much research has been carried out to improve and develop rapid detection methods for micro-organisms and/or metabolites. Developments in the areas of immunology, molecular biology, automation and computer technology occur at a rapid pace, and can contribute to more rapid, more sensitive and user-friendly methods for the food industry. In a recent study, various microbiological methods were used to monitor the development of micro-organisms during thermization and pasteurization of milk. The results show that some of the methods currently available offer possibilities for application as an 'emergency brake'. The main problem is the sensitivity of the techniques. Levels of 10^4 to 10^5 micro-organisms per ml can be measured, but this is not sufficient to adjust a production process. To permit routine in-process measurements in a production setting, the operation of the equipment will have to be simplified. Monitoring volatile metabolites of micro-organisms by headspace analysis is under development for mastitis organisms, and this technique can likely be extended to other contaminants in the future.

Control and optimization of production processes are of great importance to the food industry as this may lead to improvement of products and processes, and to cost savings. Integrated process and product development by applying rapid detection methods for critical process parameters and predictive models, therefore, is a challenge to the food industry.

In the future (objective) process control systems can be developed by integrating results of (microbiological) analyses and predictive models into process control software. In this way it is possible to adjust processes more efficiently and to respond to deviations more quickly. Computer control, neural networks and fuzzy logic may also be useful to this end.

7.5 Sources of further information and advice

http://www.cdc.gov/outbreaknet/surveillance_data.html
http://www.nsf.org/
http://www.ehedg.org/
http://www.3-a.org/
http://www.fil-idf.org/
http://www.europa.eu.int/eur-lex/

7.6 Bibliography

3-A SANITARY STANDARDS COMMITTEES (1995) *Model Document for Preparing 3-A Sanitary Standards and 3-A Accepted Practices*, 2nd edn. 3-A Sanitary Standards Committees, Dairy and Food Industries Supply Association, McLean, VA.
ANON. (1992) *Bacillus cereus* in milk and milk products. *Bulletin of the International Dairy Federation* 275, 46 pp.

ANON. (2009) The Community Summary Report on Food-borne Outbreaks in the European Union in 2007. *EFSA Journal*, 271.

AUSTIN JW and BERGERON G (1995) Development of bacterial biofilms in dairy processing lines. *J. Dairy Res.* 62: 509–519.

BOUMAN S, LUND DB, DRIESSEN FM and SCHMIDT DG (1982) Growth of thermoresistant streptococci and deposition of milk constituents on plates of heat exchangers during long operating times. *J. Food Prot.* 45: 806–812.

BURGESS K, HEGGUM C, WALKER S and VAN SCHOTHORST M (1994) Recommendations for the hygienic manufacture of milk and milk based products. *Bulletin of the International Dairy Federation* 292, 32 pp.

CHAMPAGNE CP, LAING RR, ROY D, MAFU AA and GRIFFITHS MW (1994) Psychrotrophs in dairy products: their effects and their control. *Crit. Rev. Food Sci. Nutr.* 34: 1–30.

ENEROTH A, AHRNE S and MOLIN G (2000) Contamination routes of Gram-negative spoilage bacteria in the production of pasteurised milk, evaluated by randomly amplified polymorphic DNA (RAPD). *Int. Dairy J.* 10: 325–33.

FLINT SH, BREMER PJ and BROOKS JD (1997) Biofilms in dairy manufacturing plant – description, current concerns and methods of control. *Biofouling* 11: 81–97.

GOOSEN ESM, NOTERMANS SHW and BORGDORFF MW (1997) Investigations of foodborne diseases by food inspection services in the Netherlands, 1991–1994. *J. Food Prot.* 60: 442–446.

GRUETZMACHER TJ and BRADLEY RL (1999) Identification and control of processing variables that affect the quality and safety of fluid milk. *J. Food Prot.* 62: 625–631.

HETTINGA KA, VAN VALENBERG HJF and VAN HOOIJDONK ACM (2008) Quality control of raw cows' milk by headspace analysis. *Int. Dairy J.* 18: 506–513.

HETTINGA KA, VAN VALENBERG HJF, LAM TJGM and VAN HOOIJDONK ACM (2009) The origin of the volatile metabolites found in mastitis milk. *Veterinary Microbiology* 137: 384–387.

KNEIFEL W and KASER A (1994) Microbiological quality parameters of packaging materials used in the dairy industry. *Archiv für Lebensmittelhygiene* 45: 25–48.

LANGEVELD LPM, VAN MONTFORT-QUASIG RMGE, WEERKAMP AH, WAALEWIJN R and WEVER JS (1995) Adherence, growth and release of bacteria in a tube heat exchanger for milk. *Neth. Milk Dairy J.* 49: 207–220.

LEWIS SJ and GILMOUR A (1987) Microflora associated with the internal surfaces of rubber and stainless steel milk transfer pipeline. *J. Appl. Bacteriol.* 62: 327–333.

MARCO M and WELLS-BENNIK MHJ (2008) Impact of bacterial genomics on determining quality and safety in the dairy production chain. *Int. Dairy J.* 18: 486–495.

MARTH EH and STEELE JL (1998) *Applied Dairy Microbiology.* Marcel Dekker, New York.

MEIJERING A, HOGEVEEN H and DE KONING CJAM (EDS) (2002) *Automatic Milking: A Better Understanding.* 525 p. Dairy Science Handbook from C.H.I.P.S., Texas.

OLSEN PJ, MACKINON LC, GOULDING JS, BEAN NH and SLUTSKER L (2000) Surveillance for foodborne disease outbreaks – United States, 1993–1997. *MMWR* 49 (SS01): 1–51.

PIRTTIJÄVI TSM, GRAEFFE TH and SALKINOJA-SALONEN MS (1996) Bacterial contaminants in liquid packaging boards: assessment of potential for food spoilage. *J. Appl. Bacteriol.* 81: 445–458.

SCHELDEMAN P, PIL A, HERMAN L, DE VOS P and HEYNDRICKX P (2005) Incidence and diversity of potentially highly heat-resistant spores isolated at dairy farms. *Appl. Environm. Microbiol.* 71: 1480–1494.

SCOTT S, BROOKS JD, RAKONJAC J, WALKER KMR and FLINT SH (2007) The formation of thermophilic spores during the manufacture of whole milk powder. *Int. J. Dairy*

Technol. 60: 109–117.

SVENNERSTEN-SJAUNJA KM and PETTERSSON G. (2008) Pros and cons of automatic milking in Europe. *J. Anim Sci.* 86: 37–46.

TE GIFFEL MC, BEUMER RR, LANGEVELD LPM and ROMBOUTS FM (1997) The role of heat exchangers in the contamination of milk with *Bacillus cereus* in dairy processing plants. *Int. J. Dairy Technol.* 50: 43–48.

VISSERS MMM and DRIEHUIS F (2009) On-farm hygienic milk production. In: *Milk Processing and Quality Management* (ed. Tamime AY), Blackwell, Oxford, 1–22.

VISSERS MMM, TE GIFFEL MC, DRIEHUIS F, DE JONG P and LANKVELD JMG (2007) Predictive modeling of *Bacillus cereus* spores in farm tank milk during grazing and housing periods. *J. Dairy Sci.* 90: 281–292.

VISSERS MMM, TE GIFFEL MC, DRIEHUIS F, DE JONG P and LANKVELD JMG (2007) Minimizing the level of *Bacillus cereus* spores in farm tank milk. *J. Dairy Sci.* 90: 3286–3293.

8

Exploiting genetic variation in milk-fat composition of milk from dairy cows

J. A. M. van Arendonk, H. J. F. van Valenberg and H. Bovenhuis, Wageningen University and Research Centre, The Netherlands

Abstract: Milk fat contains many nutrients necessary for humans, including fat-soluble vitamins, energy, and bioactive lipids. It is important to understand the genetic basis for milk-fat composition in cows' milk. Knowledge of these genetic parameters can be used to predict how different traits will respond to genetic selection. The predicted response in traits, including trade-offs between different traits, plays an important role in designing breeding schemes in dairy cattle. This chapter reviews the genetic variation in milk-fat composition between cows and looks into the genetic correlations with other traits.

Key words: milk-fat composition, genetic parameters, cattle breeding.

8.1   Introduction

Milk fat contains many nutrients necessary for humans, including fat-soluble vitamins, energy, and bioactive lipids (German and Dillard, 2006). Hulshof *et al.* (1999) showed that milk and milk-derived foods (including cheese and butter) were the main source of dietary saturated fatty acids across Europe; milk and milk-derived foods contributed for 27–58% to the intake of saturated fatty acids in the diet. The contribution from milk and milk-derived foods to dietary *trans* fatty acids was between 17% and 72%. Milk fat is relatively high in saturated fatty acids, especially C14:0 and C16:0, and low in polyunsaturated fatty acids. Recently, milk fat consumption has become negatively associated with human health (Bitman *et al.*, 1995; Jensen, 2002). The impact of dietary fat on chronic disease, such as coronary heart disease, has been a topic of interest for decades

(Hu and Willett, 2002). For almost 50 years, effects of fatty acid intake on serum lipids have been investigated. It is now clear that intake of saturated fatty acids increases total and low-density lipoprotein (LDL) cholesterol levels, whereas intake of polyunsaturated fatty acids decreases LDL cholesterol (Mensink *et al.*, 2003). Not all saturated fatty acids affect cholesterol concentrations to the same extent. Lauric acid (C12:0), for example, reduces the ratio of total to high-density lipoprotein (total:HDL) cholesterol; reduction in this ratio is associated with reduction in the risk of coronary heart disease. Myristic acid (C14:0) and stearic acid (C18:0), furthermore, reduce total:HDL cholesterol less than C12:0, whereas palmitic acid (C16:0) has the unfavourable effect of increasing the ratio (Mensink *et al.*, 2003). These findings suggest that an alteration of the dietary fat composition could have a major impact on public health.

Milk-fat composition can be altered through the nutrition of dairy cows (e.g. Palmquist, 2006), and possibly by selective breeding, although prospects for the latter have not been studied extensively. The major prerequisite for selective breeding is the existence of genetic variation in milk-fat composition among cows. For milk-fat percentage, around half of the observed variation between cows is estimated to be due to genetic variation. Phenotypic variation in milk-fat composition has been reported as well, both between and within breeds, although the number of reports on genetic variation is limited (Stull and Brown, 1964; Renner and Kosmack, 1974b; Karijord *et al.*, 1982; Lawless *et al.*, 1999; Soyeurt *et al.*, 2006).

In the Netherlands, selection on milk-production traits has contributed to an increase in milk-fat percentage, from 3.66% in 1950 to 4.42% in 2005 (NRS, 2006). The consequences of this increase in milk-fat percentage on milk-fat composition are unknown. Knowledge on genetic correlations between milk-fat percentage and milk-fat composition is needed for evaluating these consequences. In more detail, knowledge on genetic parameters can be used to predict how different traits will respond to genetic selection. The predicted response in traits, including trade-offs between different traits, plays an important role in designing breeding schemes in dairy cattle. In this chapter we will, therefore, first report on the genetic variation between cows in milk-fat composition and subsequently look into the genetic correlations with other milk production traits.

In recent decades, genetic improvement of farm animals has contributed to meeting the needs of the growing world population. Genetic improvement programmes exploit genetic variation among animals. Genetic variation has been found in most traits (production, reproduction and health) investigated in livestock species. Until recently, little was known of the genetic basis of this variation. For most purposes it is assumed that genetic differences are caused by a large number of genes, each having a small effect. Selection procedures based on this genetic model have been successfully applied in practice: animals are selected on their predicted breeding value, The prediction of breeding values is based on the phenotype for the trait recorded on the animal itself and/or those of its relatives and uses information on heritability of the trait and similarity between relatives. Breeding values are predicted without knowing the genes that

are responsible. Over the past few years, we have seen spectacular advancements in molecular genetics. The release of the first draft of the cattle genome in 2004 offered researchers new tools for analysing the bovine genome to uncover more information about individual genes and their effect on important traits in cattle. Knowledge on genes can be particularly beneficial for traits that are difficult to record, which includes milk quality traits (e.g. Georges *et al.*, 1995; Spelman *et al.*, 1999). In this chapter, we will report on opportunities to use molecular genetics to identify differences among individuals at the genotypic level rather than the phenotypic level.

8.2   The Dutch Milk Genomics Initiative

The objective of the Dutch Milk Genomics Initiative was to increase our understanding of variation between cows in milk composition in order to explore opportunities to alter milk composition through breeding. For estimating genetic parameters (heritabilities and genetic correlations), a design with a large number of relatively small number of individuals per family is most informative. On the other hand, for linkage mapping of genes that contribute to genetic variation, a design with a small number of families with a relatively large number of individuals per family is most informative. To be able to estimate genetic parameters and to map genes, we aimed for collection of data on approximately 2000 cows descending from a number of selected bulls; 50 young bulls were aimed to have 20 daughters each, and five proven bulls were aimed to have 200 daughters each. In selecting the cows to be included in the study, only farms that milked twice a day were selected, the minimum number of selected cows on each farm was three, all cows needed to be in their first lactation, and all cows needed to be in the first part of lactation in order to be able to collect milk from the same cows in winter and summer. In order to meet the minimum number of cows on each farm, daughters from other proven bulls were included. The first milk sample was collected in February and March (winter) of 2005 from 1918 first-lactation cows on 398 commercial herds. These cows descended from one of 50 young bulls (843 cows), from one of five proven bulls (888 cows) or from other proven bulls (187 cows) (for further details see Stoop *et al.*, 2008). Each cow was more than 87.5% Holstein–Friesian, and was between day 63 and day 263 in milk during their first lactation. Cows were milked twice daily, but only the morning milk was collected for the study to ensure the quality of the samples. Milk was cooled to 4°C within 3 h after sampling and transported to the laboratory the same morning. Sample bottles contained sodium azide (0.03 w/w%) for conservation. The first milk sample of 500 mL per cow was collected between February and March 2005 (winter). The second milk sample of 500 mL per cow was collected from the same cows, provided they were not removed from the herd, between May and June 2005 (summer).

8.2.1   Analysis of milk samples

Milk fat (butter) was extracted from approximately 400 mL of milk, keeping the remaining 100 mL for other analyses. Fatty acid methyl esters were prepared from milk fat as described in ISO Standard 15884 (ISO-IDF, 2002b). The methyl esters were analysed by gas chromatography according to the 100% FA methyl ester method (ISO-IDF, 2002a) with a 100 m polar column (Varian Fame Select CP 7420, Varian Inc., Palo Alto, CA) at the laboratory of the Netherlands Controlling Authority for Milk and Milk Products (Leusden, the Netherlands). The FA were identified and quantified by comparing the methyl ester chromatograms of the milk-fat samples with the chromatograms of pure FA methyl ester standards, and were measured as the weight proportion of total fat weight. The chromatograms resulted in approximately 130 measurable FA peaks, of which approximately one-third could be identified. Of these, 16 major FA were used for the genetic analysis: the even-numbered FA C4:0 to C18:0; five identified C18:1 isomers; C18:2 *cis*-9,12; C18:3 *cis*- 9,12,15; and conjugated linoleic acid (CLA) *cis*-9, *trans*-11. These 16 FA comprised 89% of the total fat. In addition to the individual FA, a number of FA groups were defined based on their potential effect on human health (German and Dillard, 2006): a 'neutral' group (C6–12) containing C6:0, C8:0, C10:0 and C12:0; a 'negative' group (C14–16) containing C14:0 and C16:0; and a 'positive' group (C18u) containing all unsaturated C18 that were part of the data set. Data were analysed as weight proportion. To calculate the ratio of SFA to UFA, 11 additional mono-unsaturated FA and odd-chain FA were included. Percentages of fat and protein were determined from a 10 mL milk subsample by infrared spectroscopy by using a Fourier-transformed interferogram (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark) at the certified laboratory of the Milk Control Station (Zutphen, the Netherlands). The NRS supplied the corresponding morning test-day milk yield of the samples. In total, 1918 winter samples and 1689 summer samples were analysed for fat and protein percentages and fat composition. Unless stated otherwise, results in this chapter refer to the winter sample.

8.3   Mean milk-fat composition in winter and summer

Several studies have reported variation in milk-fat composition over the year (Karijord *et al.*, 1982; Palmquist and Beaulieu, 1993; Heck *et al.*, 2009). In general it seems that proportions of short-chain FA, C6:0 to C16:0, are increased during the winter, whereas long-chain FA, C18:1 and C18:3, are higher in summer than in winter. Palmquist and Beaulieu (1993) suggested that all seasonal effects on milk-fat composition are presumably caused by dietary changes between seasons, with a large effect of fresh grass availability.

To study seasonal variation in milk composition, Heck *et al.* (2009) analysed weekly bulk milk samples representative for the complete Dutch milk supply from February 2005 until February 2006. They found the largest seasonal variation in the *trans* fatty acids. Based on the seasonal pattern, two groups of

fatty acids could be distinguished, viz. fatty acids that have a minimum in the summer and a maximum in the winter, and fatty acids that have a minimum in the winter and a maximum in the summer. In general, the fatty acids that have a minimum in the summer are the fatty acids that are synthesized *de novo*, while the fatty acids that have a minimum in the winter are blood-derived fatty acids. Milk C16:0 originates for some 50% from arterial blood and 50% from *de novo* synthesis (Barber *et al.*, 1997), and this fatty acid was also lowest in the summer period. Similar seasonal patterns, i.e. lower values of *de novo* synthesized and higher values for the blood-derived fatty acids in the summer, have been reported in French, German (Precht and Molkentin, 1999), American (Palmquist and Beaulieu, 1993), and Swiss (Collomb *et al.*, 2008) milk. In New Zealand, rather different seasonal patterns in fatty acids have been observed (Auldist *et al.*, 1998). New Zealand dairy farming is mainly based on the use of pasture and seasonal calving is adopted to maximize pasture utilization. France and Germany use similar feeding strategies throughout the season as the Netherlands and also do not have a strong seasonal calving pattern.

Mean and coefficient of variation for milk-fat composition during winter and summer samples collected in the Dutch Milk Genomics Initiative are shown in Table 8.1. Part of the observed phenotypic changes may be due to other effects than season. The milk-fat composition was measured on the same cows, and consequently cows in the winter were in an earlier stage of their lactation than cows in the summer. Average lactation stage was 164 days for the winter sample

Table 8.1 Phenotypic means (w/w%) and coefficient of variation (CV, %) for fat content, fatty acids and fatty acid groups in winter and summer, based on 3378 records from 1689 cows

Trait	Winter		Summer	
	Mean	CV	Mean	CV
Fat (%)	4.35	16	4.27	17
C4:0	3.50	8	3.52	10
C6–12	10.76	11	10.15	13
C14:0	11.63	8	11.16	9
C16:0	32.58	9	29.19	12
C18:0	8.71	16	9.88	18
C5–15	1.47	22	1.33	22
C18 *trans*	1.50	29	2.14	34
SFA[a]	71.03	4	68.08	6
UFA[a]	26.00	10	28.85	12
Ratio SFA/UFA[a]	2.77	13	2.41	17
Total index[a]	0.27	11	0.30	13
CLA *cis*-9, *trans*-11	0.40	28	0.56	50
C18:2 *cis*-9,12	1.21	24	1.12	22
C18:3 *cis*-9,12,15	0.41	27	0.50	32

[a] SFA = saturated fatty acids; UFA = unsaturated fatty acids; total index = total unsaturation index.
Source: Stoop *et al.* (2009c).

and 249 days for the summer sample. Fat percentage was approximately 4.3% in both winter and summer. The largest change due to season was a 3.39 w/w% reduction in C16:0 from winter to summer. C18tr, CLA cis-9, trans-11, and C18:3 cis-9,12,15 also showed large differences between winter and summer. Proportions of C6:0 to C16:0 were lower in summer than in winter, whereas proportions of long-chain FA (>18ºC), with the exception of C18:2 cis-9,12, were higher in summer. The total FA in Table 8.1 made up approximately 89% of the total fat; the remaining 11% consisted of a large number of FA present in smaller amounts. The ratio of saturated FA (SFA) to UFA averaged 2.8, indicating that approximately 71% of the fat produced in the winter was saturated. This number slightly overestimates the true ratio of SFA to UFA, because the ratio was based on only 27 major FA, whereas trace amounts of some FA, mainly UFA, were not taken into account..

The coefficients of variation for individual FA ranged from 7% for C6:0 to 28% for C18:2 (Table 8.1). A low coefficient of variation (approximately 10%) was found for most saturated FA (C4:0 to C18:0), and a higher coefficient of variation (approximately 25%) was found for most unsaturated C18 FA. The highest coefficient of variation was found for long-chain polyunsaturated FA: 28% for C18:2, and 27% for C18:3. The coefficient of variation for the entire C18u group was only 11%. Standard deviations of FA in summer were on average 20% larger than in winter, implying more variation in FA proportions among cows in summer.

8.4   Genetic variation between cows

Genetic variation (between or within breeds) allows the composition of milk to be altered through selective breeding. In this chapter we concentrate on genetic variation within a breed. Heritability (h^2) estimates the fraction of total variation within a breed that is caused by genetic differences. For fat production (expressed in kg per cow per day), heritabilities are around 30% while estimates for fat content (% of fat in the milk) are higher at 50%.

Soyeurt et al. (2006), in studying 600 milk samples from 275 animals of five breeds, found differences in FA proportions among breeds of cattle. Lawless et al. (1999) studied approximately 25 animals per breed and found a relatively high amount of C16:0 and a slightly lower amount of C18:0 in Holstein–Friesians, compared with Normande and Montbeliarde breeds. Renner and Kosmack (1974b) studied 243 cows originating from 10 sires and found evidence for within-breed genetic variation in milk-fat composition. Karijord et al. (1982) and Famula et al. (1995) also estimated within-breed genetic variation for milk fat composition. The study by Karijord et al. (1982) consisted of approximately 3000 animals and 7000 samples, and Famula et al. (1995) studied 523 animals and one sample per animal. Heritabilities for individual FA and groups of FA found in winter samples in the Dutch Milk Genomics Initiative (Stoop et al., 2008) are shown in Table 8.2. High heritabilities of 0.42 to 0.71

Table 8.2 Genetic, residual and herd variation, heritabilities, proportion of variance explained by herd, and ratio of genetic over herd variance for individual fatty acids, groups of fatty acids and milk production traits, measured on a test-day morning milk sample of 1918 cows in first lactation

Trait	σ_A^2	σ_E^2	σ_{herd}^2	Heritability[a] h^2	Herd[b]	$\sigma_A^2/\sigma_{herd}^2$
C4:0	0.027	0.038	0.013	0.35	0.17	2.09
C6:0	0.011	0.012	0.004	0.39	0.16	2.39
C8:0	0.010	0.006	0.004	0.48	0.20	2.42
C10:0	0.107	0.044	0.047	0.54	0.24	2.30
C12:0	0.177	0.106	0.217	0.35	0.43	0.81
C14:0	0.433	0.296	0.157	0.49	0.18	2.75
C16:0	2.458	3.296	2.298	0.31	0.29	1.07
C18:0	0.374	1.218	0.382	0.19	0.19	0.98
C18:1 *cis*-9	0.770	2.340	1.204	0.18	0.28	0.64
C18:1 *cis*-11	0.001	0.005	0.005	0.12	0.43	0.29
C18:1 *trans*-4-8	0.0004	0.0008	0.0013	0.18	0.49	0.35
C18:1 *trans*-9	0.0001	0.0004	0.0005	0.11	0.50	0.21
C18:1 *trans*-11	0.005	0.013	0.023	0.12	0.55	0.22
C18:2 *cis*-9,12	0.010	0.029	0.040	0.13	0.51	0.25
C18:2 *cis*-9, *trans*-11 (CLA)	0.003	0.004	0.006	0.21	0.49	0.43
C18:3 *cis*-9,12,15	0.001	0.003	0.007	0.09	0.64	0.14
Ratio SFA/UFA[c]	0.027	0.071	0.038	0.20	0.28	0.71

[a] $h^2 = \sigma_A^2/(\sigma_A^2 + \sigma_E^2)$, SE between 0.07 and 0.12.
[b] Herd $= \sigma_{herd}^2/(\sigma_A^2 + \sigma_{herd}^2 + \sigma_E^2)$, SE around 0.03.
[c] SFA = saturated fatty acids; UFA = unsaturated fatty acids.
Source: Stoop *et al.* (2008).

were found for C4:0 to C16:0. Heritabilities of 0.22 to 0.35 were found for both saturated and unsaturated C18 FA, but for CLA *cis*-9, *trans*-11, heritability was 0.42. Heritabilities for the groups C6–12 (0.67) and C18u (0.26) were in line with the results for individual FA. For C14–16, heritability was rather low (0.16) compared with heritabilities of 0.59 for C14:0 and 0.43 for C16:0. Standard errors of heritability estimates were between 0.07 and 0.12.

Renner and Kosmack (1974b) and Karijord *et al.* (1982) estimated heritabilities for short-chain FA between 0.13 and 0.26, for medium-chain FA between 0.06 and 0.11, and for unsaturated C18 at 0.04 (Renner and Kosmack, 1974b), which were lower than estimates found in our study. Both studies (Renner and Kosmack, 1974b; Karijord *et al.*, 1982) also found low heritabilities for milk yield (0.36 and 0.09) and fat percentage (0.28 and 0.09). Heritability for C4:0 to C14:0 (approximately 0.60) was higher than for unsaturated C18 (approximately 0.25), which is in agreement with the findings of Renner and Kosmack (1974b) and Karijord *et al.* (1982).

Information on FA composition was collected on close to 400 herds. This offered the opportunity to quantify the variance in FA composition explained by herds. The herd variance for the different FA, expressed as proportions of the total phenotypic variance, is also shown in Table 8.2. There can be many reasons

for variance attributable to herd (Jensen, 2002); feed differences among farms, but also other management factors, might play a role. Variance attributable to herd was lower for saturated FA (C4:0 to C18:0, approximately 0.20), than for unsaturated C18 FA (approximately 0.50). This difference, however, was not found when comparing different groups of FA: for C6–12, herd explained 27% of the variation, whereas for C18u, herd explained 31%. The proportion of variance attributable to herd is smaller for C4:0 to C14:0 (approximately 0.25) than for unsaturated C18 FA (approximately 0.50). Possible reasons for this could be that short-chain FA are synthesized *de novo* by the cow. Long-chain FA, however, originate predominantly from dietary FA, and because plant material contains mainly long-chain FA, differences in diet affect long-chain FA more than short-chain FA. The difference caused by herd effects, however, was less clear in the FA groups, where herd consistently explained approximately 30% of the variance.

8.4.1   Effects of season on genetic parameters

Heritabilities and variation due to herd for FA in winter and summer are shown in Table 8.3. The analysis revealed that heritabilities were very comparable in winter and summer. There was, however, a decrease in heritability for C6–12 from 0.67 to 0.51 and for CLA *cis*-9, *trans*-11 from 0.44 to 0.27, and an increase in heritability for C18:2 *cis*-9,12 from 0.26 to 0.39. Variation due to herd (Table

Table 8.3   Heritability (h^2) and herd variation (Herd) in winter and summer samples, phenotypic (r_p) and genetic (r_g) correlations between winter and summer samples, and correlations (r_{herd}) between herd effects in winter and summer, based on 3378 records of 1689 cows in Dutch Milk Genomics data

Trait	Winter		Summer		Correlations		
	h^2	Herd	h^2	Herd	r_P	r_A	r_{herd}
Fat (%)	0.51	0.07	0.60	0.12	0.64	0.97	0.22
C4:0	0.41	0.16	0.36	0.25	0.51	0.95	0.29
C6–12	0.67	0.27	0.51	0.23	0.55	0.94	0.39
C14:0	0.60	0.19	0.60	0.34	0.57	0.93	0.36
C16:0	0.38	0.31	0.36	0.53	0.57	0.78	0.20
C18:0	0.23	0.22	0.18	0.31	0.48	0.84	0.31
C5–15	0.28	0.28	0.30	0.31	0.56	0.98	0.42
C18 *trans*	0.23	0.48	0.29	0.66	0.38	0.58	0.28
SFA[a]	0.30	0.30	0.31	0.45	0.51	0.79	0.22
UFA[a]	0.27	0.30	0.30	0.43	0.50	0.79	0.19
Ratio SFA/UFA[a]	0.28	0.31	0.29	0.45	0.51	0.78	0.17
CLA *cis*-9, *trans*-11	0.44	0.51	0.27	0.61	0.42	0.82	0.29
C18:2 *cis*-9,12	0.26	0.52	0.39	0.59	0.60	0.96	0.76
C18:3 *cis*-9,12,15	0.24	0.64	0.22	0.65	0.48	0.83	0.40

[a] SFA = saturated fatty acids; UFA = unsaturated fatty acids.
Source: Stoop *et al.* (2009c).

8.3), however, in general was much higher in summer than in winter. For most traits, except C6–12 and C18:3 *cis*-9,12,15, variation due to herd increased approximately 50% in summer compared to winter. This suggests that the observed increase in variation in FA composition in summer compared to winter is largely due to increased herd variation. Several studies have suggested that seasonal effects are mainly caused by differences in herd management, mainly dietary changes (Palmquist and Beaulieu, 1993; Chilliard *et al.*, 2001). Elgersma *et al.* (2006) showed that the availability of fresh grass leads to milk fat with more polyunsaturated FA and more CLA *cis*-9, *trans*-11. In the current study all cows (100%) were kept inside in the winter season, whereas approximately 50% of cows had the ability for grazing in the summer season (3.5 to 24 hours per day). In the summer samples the grazing cows had approximately 4 w/w% more UFA than non-grazing cows. This supports the suggestion that variation in grazing between herds contributes to the increased herd variance in the summer.

The phenotypic, genetic and herd correlations between winter and summer FA proportions are shown in Table 8.3. The phenotypic correlations ranged from 0.38 (C18tr) to 0.64 (fat%), which indicate that observed phenotypes of a cow were moderately repeatable between winter and summer and across different stages of lactation. Genetic correlations were high, ranging from 0.78 (C16:0) to 0.98 (C5–15). An exception was the genetic correlation of 0.58 for C18tr. The high genetic correlations between winter and summer suggest that milk fat composition in winter and summer is, genetically speaking, the same trait. This is an important finding, implying that expression of the genetic merit for improved FA composition does not depend on the environmental conditions. In other words, selection of cows based on FA composition measured during the winter period will lead to a response throughout the entire year. This means that genetic selection for milk-fat composition does not need to be tailored towards season, and that selection response is independent of season of selection or season of phenotype collection.

Herd correlations were below 0.44, except for a herd correlation of 0.76 for C18:2 *cis*-9,12, indicating that herd effects in winter and summer are very different. The low herd correlations indicate that differences observed between herds during the winter can hardly be used for predicting differences during the summer period. Herds that showed, for example, high levels of UFA in winter were not necessarily the herds that had high UFA levels in summer, which resulted in low herd correlations. The low estimates of herd correlations reveal that herd management with respect to FA composition is not constant over the year.

8.4.2   Genetic correlations between FA

Genetic correlations among individual FA are shown in Table 8.4. The C4:0 had a moderate negative correlation with most other FA. The C6:0 to C14:0 FA were positively correlated (0.34 to 0.96), with a weak correlation of 0.08 for C6:0 with C14:0. The C16:0 showed negative correlations with all studied FA except

Table 8.4 Genetic correlations[a] between individual fatty acids, measured on a test-day morning milk sample of 1918 cows during first lactation in the winter period

	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1 cis-9	C18:1 cis-11	C18:1 trans-4-8	C18:1 trans-9	C18:1 trans-11	C18:2 cis-9,12	C18:3 cis-9,12,15 CLA
C6:0	0.48														
C8:0	−0.05	0.82													
C10:0	−0.40	0.55	0.92												
C12:0	−0.56	0.34	0.81	0.96											
C14:0	−0.49	0.08	0.50	0.72	0.76										
C16:0	0.52	0.15	−0.33	−0.64	−0.67	−0.84									
C18:0	0.15	−0.01	−0.23	−0.26	−0.29	−0.34	0.27								
C18:1 cis-9	−0.11	−0.58	−0.53	−0.35	−0.35	0.13	−0.37	−0.41							
C18:1 cis-11	−0.62	−0.31	0.11	0.39	0.39	0.51	−0.79	0.02	0.35						
C18:1 trans-4-8	−0.32	−0.38	−0.21	0.01	0.14	0.42	−0.51	0.05	0.25	0.47					
C18:1 trans-9	−0.49	−0.36	−0.01	0.20	0.26	0.46	−0.74	−0.44	0.63	0.59	0.66				
C18:1 trans-11	−0.12	0.05	0.07	0.09	0.13	0.12	−0.38	0.05	0.12	0.40	0.69	0.78			
C18:2 cis-9,12	−0.23	0.06	0.33	0.42	0.45	0.61	−0.79	−0.27	0.41	0.33	0.52	0.86	0.63		
C18:3 cis-9,12,15	−0.31	−0.28	−0.12	0.02	0.09	0.31	−0.61	−0.36	0.62	0.55	0.73	0.99	0.94	0.90	
C18:2 cis-9, trans-11 (CLA)	−0.14	−0.10	0.02	0.08	0.14	0.33	−0.59	−0.58	0.54	0.35	0.55	0.87	0.76	0.68	0.95

[a] SE between 0.01 and 0.26. Most SE were between 0.12 and 0.21, with strong correlations having SE between 0.01 and 0.10.
Source: Stoop *et al.* (2008).

Fig. 8.1   Cluster tree based on principal component analysis of genetic correlations among individual fatty acids. Eight clusters explain over 90% of variation (dotted line).

for C4:0, C6:0 and C18:0. The C18:0 also showed negative correlations with most other FA, but the correlations were weak. The unsaturated C18 FA were positively correlated (0.25 to 0.99), with a weak correlation of 0.12 for C18:1 *cis*-9 with C18:1 *trans*-11. A clustering tree to visualize the genetic correlations among FA is shown in Fig. 8.1. Eight clusters explained more than 90% of the variance. The figure shows the clustering of C6:0 to C14:0 in one group and the clustering of unsaturated C18 in another group. This reflects the high correlations within these groups. The C4:0 and C16:0 did not cluster in one of these two main groups. The latter might reflect that C16:0 is partly synthesized *de novo* and partly originates from dietary FA.

Few papers have studied genetic correlations between FA. Genetic correlations found in the Dutch Milk Genomics Initiative were comparable to the results reported by Karijord *et al.* (1982). Explanation for the grouping of the FA can be found in the biological pathways of synthesis. The FA C4:0 (butyric acid) was negatively correlated with almost all other FA. It is formed partly in the rumen by bacterial processes, together with acetate and propionate, and is a precursor for most other short- and medium-chain FA. Increased *de novo* synthesis will possibly convert more C4, so less C4 is present in milk, hence the negative correlation. The C6:0 to C16:0 FA are synthesized *de novo* in a FA cycle starting from C2 and C4 (Bobe *et al.*, 1999). The C16:0, however, is partly synthesized *de novo* and partly

excreted from blood, which might explain the correlations found for this FA. The unsaturated C18 FA originate mainly from dietary FA, and their proportions are highly dependent on rumen biohydrogenation and on Δ9-desaturase enzymatic activity in the mammary gland (MacGibbon and Taylor, 2006). Because of the synthesis pathways, short- and medium-chain FA are expected to be under stronger genetic control than long-chain FA. This is also reflected in the higher heritability estimates and the smaller influence of herd for short- and medium-chain FA, compared with long-chain FA.

8.4.3   Correlation between fat percentage and FA

Average milk fat percentage in the Netherlands has increased from 3.7% in 1950 to 4.4% in 2005 (NRS, 2006). In the Dutch Milk Genomics Initiative a positive genetic correlation was found between fat percentage and C16:0, and a negative genetic correlation was found between fat percentage and unsaturated C18 FA (Stoop *et al.*, 2008). As a result of the increase in fat percentage, a correlated increase in C16:0 and a decrease in unsaturated C18 FA were expected. In 1974, Renner and Kosmack (1974a) found a fat percentage of 4%, with 25.5% C16:0 and 31.1% unsaturated C18 FA. In our study, the fat percentage was 4.36%, with 32.6% C16:0 and 21.6% unsaturated C18 FA. Thus, comparing results from this study with those from Renner and Kosmack (1974a), fat percentage has increased, with a strong increase in the proportion of C16:0 and an even stronger decrease in the proportions of unsaturated C18 FA. Although there may be many reasons for these differences, such as breed, season, lactation stage, time of day or feed, the change in milk fat composition could have been a correlated response to selection for fat yield, favouring C16:0 rather than increasing all FA simultaneously.

8.4.4   Unsaturation indices

The cow's diet plays a role in determining the degree of unsaturation of milk fat (Baumgard *et al.*, 2000; Perfield *et al.*, 2006, 2007). Dietary fatty acids are hydrogenated in the rumen by bacteria and transported via the blood. In the mammary gland, fatty acids originating from the blood or from *de novo* fatty acid synthesis can be desaturated. Eventually, the fatty acids that are secreted into the milk determine the degree of unsaturation of milk fat. This degree of unsaturation is often addressed by a so-called index: the concentration of the unsaturated product as a proportion of the sum of the unsaturated product and the saturated substrate.

Studies demonstrating a significant variation in unsaturation among breeds and cows on the same diet suggest that also genetics plays a role (Lawless *et al.*, 1999; Lock and Garnsworthy, 2002). For example, Kelsey *et al.* (2003) found that the milk-fat content of C18:2 *cis*-9, *trans*-11 (conjugated linoleic acid, CLA) and the CLA index shows a more than threefold variation among individual cows on the same diet.

Fatty acid unsaturation indices were calculated from fatty acid profiles of milk collected in the winter in the Dutch Milk Genomics Initiative (Schennink *et al.*, 2008). Unsaturation indices of the short- and medium-chain fatty acids C10, C12, C14 and C16 were between 2.7 and 10.9 (Table 8.5). Indices of the long-chain fatty acids C18 and CLA were higher, 67.6 and 33.7, respectively. These values are in line with those reported for dairy cattle in other studies (Perfield *et al.*, 2006, 2007; Mele *et al.*, 2007). Almost all C10, C12 and C14, and approximately 50% of C16 fatty acids are synthesized in the mammary gland, whereas the longer-chain fatty acids as well as a proportion of the unsaturated long-chain fatty acids are derived from the blood. The dual origin of the long-chain fatty acid unsaturation may play a role in the contrast between short/medium- and long-chain fatty acid unsaturation indices. An alternative explanation for this contrast may be that long-chain fatty acids are unsaturated to a larger extent. The coefficient of variation was lowest for the C18 index (6%) and highest for the C12 and C16 indices (about 20%).

Estimates of heritability ranged from 0.23 for the CLA index to 0.46 for the C16 index and demonstrated a significant genetic effect on the variation in fatty acid unsaturation indices (Table 8.5). The heritability for the total index was 0.30. Repeatabilities for C14, C16 and C18 indices, which are considered to be the upper limit of heritabilities, were estimated between 40 and 45% by Soyeurt *et al.* (2006), suggesting moderate heritabilities as well. Only Royal and Garnsworthy (2005) reported heritabilities for fatty acid unsaturation indices, based on 1520 Holstein–Friesian cows, and reported similar values for C14 (0.30), C18 (0.19) and CLA (0.29) indices, but much lower values for C16 (0.01) and total (0.02) indices. The proportion of total variance explained by

Table 8.5   Mean, coefficient of variation (CV), additive genetic variance (σ_A^2), heritability (h^2), proportion of variance explained by herd (Herd), and ratio between additive genetic variance and herd variance ($\sigma_A^2/\sigma_{herd}^2$) for fatty acid unsaturation indices, measured on one morning milk sample from 1933 first-lactation Dutch Holstein–Friesian cows

Trait[a]	Mean	CV (%)	σ_A^2	Heritability[b] h^2	Herd[c]	$\sigma_A^2/\sigma_{herd}^2$
C10 index	10.9	17	1.23	0.37	0.06	5.0
C12 index	2.7	20	0.09	0.37	0.06	5.6
C14 index	10.5	17	1.35	0.45	0.06	6.6
C16 index	4.2	19	0.30	0.46	0.07	6.2
C18 index	67.6	6	4.36	0.33	0.06	5.1
CLA index	33.7	12	3.49	0.23	0.09	2.5
Total index	26.4	10	1.58	0.30	0.26	0.8

[a] Indices are calculated according to the following example: C14 index = [C14:1 *cis*-9/(C14:1 *cis*-9 + C14:0)] × 100; CLA index = [CLA *cis*-9, *trans*-11/(CLA *cis*-9, *trans*-11 + C18:1 *trans*-11)] × 100.
[b] $h^2 = \sigma_A^2/(\sigma_A^2 + \sigma_E^2)$, SE between 0.07 and 0.09.
[c] Herd $= \sigma_{herd}^2/(\sigma_A^2 + \sigma_{herd}^2 + \sigma_E^2)$, SE around 0.02.
Source: Schennink *et al.* (2008).

herd was small, ranging from 0.06 to 0.09 of total variance in individual unsaturation indices. For the total unsaturation index the herd effect was larger (0.26). The ratio of genetic variance to herd variance showed that for all indices the genetic variance was much larger than the herd variance, except for the total index, for which the herd variance was slightly larger than the genetic variance. The total unsaturation index, in fact, mainly represents the ratio of C18:1 *cis*-9 to C16:0 because these fatty acids are the largest unsaturated and saturated fatty acid fractions in milk. The proportion of variance explained by herd was 0.28 for the C18:1 *cis*-9 fraction and 0.29 for the C16:0 fraction in milk, which explains the relatively large herd variance of the total unsaturation index (Stoop *et al.*, 2008). The moderate to high heritabilities for unsaturation indices in combination with the moderate to high coefficients of variation indicate that the unsaturation indexes can be changed by means of selection.

8.5   Molecular genetics

8.5.1   Role of diacylglycerol acryltransferase 1 (DGAT1)

Traditional selective breeding requires extensive recording of phenotypes. Conversely, a direct handle on the genes conferring merit enables faster genetic progress. Recently, a quantitative trait locus (QTL) mapping study in cattle resulted in the identification of the K232A mutation in the gene coding for acyl CoA:DGAT1, which is a key enzyme in triglyceride synthesis (Cases *et al.*, 1998) and has a strong effect on milk-fat percentage and other milk-production characteristics (Grisart *et al.*, 2002; Winter *et al.*, 2002). Female mice deficient in DGAT1 do not produce milk (Smith *et al.*, 2000) and show an altered fatty acid composition in adipose tissue and skeletal muscle: less monounsaturated C16:1 and C18:1 and more saturated C16:0 and C18:0 fatty acids (Chen *et al.*, 2002). Using a mathematical model of fatty acid synthesis and triglyceride assembly, Shorten *et al.* (2004) predicted that an increase in milk yield due to the DGAT1 232K allele would lead to a more saturated fat composition. Schennink *et al.* (2007) showed that the K allele of DGAT1 is associated with a higher ratio of saturated to unsaturated fatty acids, a higher proportion of C16:0 and a lower proportion of C14:0 (Table 8.6). Estimated effects of the DGAT1 K232A mutation on fat percentage, protein percentage and yield traits are consistent with previous studies (Grisart *et al.*, 2002; Spelman *et al.*, 2002; Thaller *et al.*, 2003). The K allele increases fat percentage, protein percentage and fat yield, whereas it decreases milk yield and protein yield. Interestingly, the K allele leads to an increase in the fraction of C16:0 and the SFA/UFA ratio, whereas it leads to a decrease in the fractions of C14:0, unsaturated C18 and CLA (Table 8.6). The DGAT1 K232A mutation explains large proportions of the genetic variance: 50% for fat percentage, 53% for unsaturated C18, 40% for C16:0 and 36% for SFA/UFA. Effects of DGAT1 on fat composition are in line with expectations based on the effect of DGAT1 on fat percentage and the genetic correlations between fat percentage and fat composition (Table 8.4).

Table 8.6   Effect of the DGAT1 K232A polymorphism on fatty acid composition

Trait	KK (n = 289)	KA[a] (SE) (n = 829)	AA[b] (SE) (n = 644)	P-value[c]	$r^2_{genetic}$ (%)[d]
Fat (%)	0	−0.45 (0.04)	−0.98 (0.04)	<0.001	50
C4:0–C12:0	0	0.16 (0.07)	0.03 (0.08)	0.05	1
C14:0	0	0.43 (0.06)	0.79 (0.06)	<0.001	23
C16:0	0	−1.02 (0.16)	−2.52 (0.17)	<0.001	40
C18:0	0	−0.18 (0.09)	−0.10 (0.10)	0.18	1
C18u	0	0.80 (0.14)	2.12 (0.15)	<0.001	53
CLA	0	0.02 (0.01)	0.05 (0.01)	<0.001	16
Trans	0	−0.01 (0.02)	0.04 (0.03)	0.03	2
Ratio SFA/UFA[e]	0	−0.11 (0.02)	−0.27 (0.02)	<0.001	36

[a] Contrast of KA–KK genotypes.
[b] Contrast of AA–KK genotypes.
[c] Statistical significance of the DGAT1 K232A effect.
[d] Percentage of the genetic variance explained by the DGAT1 K232A polymorphism.
[e] SFA = saturated fatty acids; UFA = unsaturated fatty acids.

DGAT1 catalyses the last step in triglyceride synthesis: the esterification of a fatty acyl-CoA to the sn-3 position of a diacylglycerol. The effect of the DGAT1 K232A mutation on fat composition may have different causes: a higher activity of DGAT1 and alteration of specificity of DGAT1.

8.5.2   Genes involved in desaturation

Animals are capable of desaturating saturated fatty acids to Δ9 unsaturated fatty acids by the stearoyl-CoA desaturase (SCD) enzyme, which catalyses the insertion of a double bond between carbon atoms 9 and 10 of a fatty acid (Pereira *et al.*, 2003). Two SCD isoforms have been identified in cattle, SCD1 and SCD5; SCD1 is located on chromosome 26 and expressed in a variety of tissues among which are adipose and mammary tissue, and SCD5 is located on chromosome 6 and expressed primarily in the brain (Chung *et al.*, 2000; Lengi and Corl, 2007). A non-synonymous SNP in exon 5 of SCD1, causing the substitution of valine with alanine (A293V), has been associated with carcass fatty acid composition in Japanese Black cattle (Taniguchi *et al.*, 2004) and with milk fatty acid composition in Italian Holstein, Piedmontese and Valdostana cattle (Mele *et al.*, 2007; Moioli *et al.*, 2007). The SCD1 A allele was associated with a higher monounsaturated fatty acid content. Another candidate gene that may affect unsaturation is acyl CoA:DGAT1, which is located on chromosome 14 (Grisart *et al.*, 2002). Schennink *et al.* (2008) showed that both the SCD1 A293V and the DGAT1 K232A polymorphism explain part of the genetic variation in the unsaturation indices of milk fat. Even though the SCD1 A293V polymorphism does not affect the overall degree of unsaturation, its effects on the individual fatty acid indices offer opportunities for improving milk fatty acid composition. Their study gives more insight into the process of unsaturation.

Not only does SCD1 play a significant role by desaturating saturated fatty acids into unsaturated fatty acids, but also DGAT1 is important by influencing the composition of the triacylglycerols. The entire pathway of lipogenesis, which next to SCD1 and DGAT1 involves other enzymes such as fatty acid synthase and acetyl-coA carboxylase, is regulated by the transcription factor sterol regulatory element-binding protein (SREBP)-1c and activated by dietary fatty acids. Detection of genes that contribute to the genetic variation of unsaturation can be the start of other studies looking into the interaction between genetics and feeding.

8.5.3   Whole genome scan

In the previous sections, we have reported on the effects of so-called candidate genes on milk fat composition. In case of the candidate-gene approach, involvement of a gene in a particular trait is postulated based on knowledge of the underlying physiology or knowledge from other species. The contribution of a candidate gene to genetic variation in a trait is tested by estimating the effects of polymorphism in the coding region of that gene. A whole genome scan is an alternative strategy for the detection of genes that contribute to genetic variation in a trait. In a whole genome scan, animals are genotyped for a large number of markers on the genome. The markers are used for the detection of regions of the genome, referred to as quantitative trait loci (QTL), that harbour genes that contribute to the genetic variation. Since the initiation of whole genome scans in dairy cattle by Georges *et al.* (1995), several genome scans have been undertaken to identify the genomic regions harbouring genes that underlie genetic variation of production and conformation traits in dairy cattle. Several QTL and candidate genes for milk production, reproduction, functional and conformation traits have been identified in previous studies on *Bos taurus* autosomes (BTA) 1, 2, 3, 4, 5, 6, 7, 9, 14, 19, 20, 23, 26, 27 and 29. Some of these QTL were detected and mapped in numerous studies (Schrooten *et al.*, 2000; Boichard *et al.*, 2003; Ashwell *et al.*, 2005; Schnabel *et al.*, 2005).

Single nucleotide polymorphisms (SNPs) are the most abundant form of DNA variation in the genome and their preference for use in whole genome scans is growing due to the ease of genotyping. The efforts to sequence the bovine genome have resulted in a large set of SNPs spanning the whole bovine genome, which accelerates research on unravelling genetic variation in cattle.

QTL mapping for milk-fat composition has only been reported by Morris *et al.* (2007), who focused on a single chromosome, BTA19. Morris *et al.* (2007) detected a QTL for C18 fatty acids, and found an association between an SNP in FASN and C18:0 and C18:1 *cis*-9 in milk. Schennink *et al.* (2009a) were not able to confirm these findings. A significant effect of the FASN genotype on C18 fatty acids was also found in tissue from the longissimus muscle of beef cattle (Abe *et al.*, 2008). Different production circumstances might explain this: a pasture-based system in New Zealand vs. an indoor winter period in the Netherlands. Regarding genome-wide scans for milk fatty acid composition,

there is no comparable literature, although (partial) genome scans to detect QTL for carcass fatty acid composition have been performed in beef cattle, pigs and sheep (Abe *et al.*, 2008; Alexander *et al.*, 2007; Clop *et al.*, 2003; Karamichou *et al.*, 2006; Sanchez *et al.*, 2007). Alexander *et al.* (2007) analysed the fatty acid composition of the longissimus muscle of Wagyu × Limousin cattle, and found significant QTL on BTA2 (a.o. for MUFA, SFA, CLA and ratio of C18:1 to C18:0) and BTA7 (for MUFA). Abe *et al.* (2008) mapped QTL for fatty acid composition of the longissimus muscle of Japanese Black × Limousin cattle, and detected QTL on BTA2 (a.o. for C18:2) and on BTA19 (a.o. for C18:1).

The studies of Stoop *et al.* (2009b) and Schennink *et al.* (2009b) are, to our knowledge, the first to present results of a whole genome scan for milk-fat composition, and are an important step in the unravelling of regulation of lipogenesis of fatty acids. From the Dutch Milk Genomics population, a total of 849 cows representing five large and two small paternal half-sib families, and their seven sires, were genotyped for 1341 SNP across all autosomes. A genetic map was constructed comprising 1341 SNP and 2829 cM with an average information content of 0.83. QTL analyses were performed using a weighted cross-family regression on phenotypes, which were pre-adjusted for systematic environmental effects.

The whole-genome scan of short- and medium-chain fatty acids (Stoop *et al.*, 2009b) revealed significant evidence for QTL ($P_{genome} < 0.05$) for 23 traits on four chromosomes. Significant QTL were found for C6:0 and C8:0 on BTA6; for fat %, all uneven-chain fatty acids, and C14:0, C16:0 and C16:1 and their unsaturation indices on BTA14; for C14:0 on BTA19; and for the mono-unsaturated fatty acids and their unsaturation indices on BTA26. The QTL explained 3–19% of phenotypic variance. Furthermore, 49 traits with suggestive evidence for linkage ($P_{chromosome} < 0.05$) were found on 21 chromosomes. Additional analyses revealed that the QTL on BTA14 was most likely caused by a mutation in DGAT1, whereas the QTL on BTA26 was most likely caused by a mutation in the SCD1 gene. Schennink *et al.* (2009b) detected significant QTL ($P_{genome-wise} < 0.05$) on BTA14, 15 and 16 in their whole-genome scan of long-chain fatty acids. They detected significant QTL for C18:1 *cis*-9, C18:1 *cis*-12, C18:2 *cis*-9,12, CLA *cis*-9, *trans*-11, C18:3 *cis*-9,12,15, C18 index, total index, total saturated fatty acids (SFA), total unsaturated fatty acids (UFA) and SFA/UFA ratio on BTA14, for C18:1 *trans* fatty acids on BTA15, and for C18 and conjugated linoleic acid (CLA) indices on BTA16. The QTL explained 3–19% of the phenotypic variance in the traits. Suggestive QTL were found on 16 other chromosomes. The DGAT1 K232A polymorphism on BTA14 is known to influence fatty acid composition. This polymorphism most likely explains the QTL detected on BTA14.

Allele substitution effects for the significant QTL affecting long-chain fatty acids on BTA14, 15 and 16 are shown in Table 8.7. Families 1, 2, 3, 4 and 7 segregated for the QTL for fat percentage, C18:1 *cis*-9 and C18:3 *cis*-9,12,15 on BTA14 (Table 8.3). These families, but not necessarily all five, also contributed

Table 8.7 Allele substitution effects and SE within seven paternal half-sib families for QTL on BTA14, 15 and 16, and approximate phenotypic variation explained by the QTL

Trait	Family (no. of daughters)							Phenotypic variation explained by QTL (%)
	1 (193)	2 (179)	3 (170)	4 (166)	5 (91)	6 (29)	7 (21)	
BTA14								
Fat (%)	$-0.53_{0.09}$*	$-0.69_{0.10}$*	$-0.70_{0.11}$*	$-0.68_{0.10}$*	$-0.06_{0.19}$	$0.50_{0.34}$	$-0.93_{0.26}$*	19
C18:1 *cis*-9	$1.10_{0.23}$*	$0.60_{0.23}$*	$1.37_{0.26}$*	$1.18_{0.24}$*	$0.50_{0.47}$	$-0.26_{0.83}$	$1.83_{0.65}$*	10
C18:1 *cis*-12	$0.01_{0.01}$*	$0.01_{0.01}$*	$0.01_{0.01}$	$0.02_{0.01}$*	$0.00_{0.01}$	$0.02_{0.02}$	$0.02_{0.01}$	4
C18:2 *cis*-9,12	$0.06_{0.03}$*	$0.07_{0.03}$*	$0.06_{0.03}$	$0.13_{0.03}$*	$0.00_{0.06}$	$0.19_{0.10}$	$0.18_{0.08}$*	5
CLA *cis*-9, *trans*-11	$0.02_{0.01}$*	$0.01_{0.01}$	$0.03_{0.01}$*	$0.04_{0.01}$*	$0.04_{0.02}$	$-0.04_{0.04}$	$0.07_{0.03}$*	4
C18:3 *cis*-9,12,15	$0.03_{0.01}$*	$0.02_{0.01}$*	$0.03_{0.01}$*	$0.06_{0.01}$*	$0.01_{0.02}$	$0.01_{0.03}$	$0.05_{0.02}$*	7
C18 index	$1.55_{0.56}$*	$0.36_{0.56}$	$2.43_{0.63}$*	$0.78_{0.59}$	$1.47_{0.14}$	$-1.45_{2.00}$	$4.19_{1.56}$*	4
Total index	$0.83_{0.25}$*	$0.32_{0.25}$	$1.27_{0.28}$*	$0.82_{0.26}$*	$0.77_{0.51}$	$-0.51_{0.90}$	$1.60_{0.70}$*	6
SFA	$-1.18_{0.32}$*	$-0.51_{0.33}$	$-1.58_{0.37}$*	$-1.36_{0.34}$*	$-0.95_{0.66}$	$0.29_{1.17}$	$-2.42_{0.91}$*	7
UFA	$1.04_{0.30}$*	$0.48_{0.30}$	$1.42_{0.33}$*	$1.17_{0.31}$*	$0.84_{0.60}$	$-0.30_{1.10}$	$2.03_{0.83}$*	6
Ratio SFA/UFA	$-0.15_{0.04}$*	$-0.09_{0.04}$	$-0.21_{0.05}$*	$-0.18_{0.05}$*	$-0.12_{0.09}$	$0.03_{0.16}$	$-0.33_{0.12}$*	7
BTA15								
C18:1 *trans*	$-0.19_{0.04}$*	$-0.08_{0.04}$*	$0.01_{0.04}$	$0.02_{0.04}$	$-0.01_{0.06}$	$-0.01_{0.09}$	$0.10_{0.11}$	4
BTA16								
C18 index	$1.60_{0.54}$*	$0.22_{0.53}$	$2.61_{0.76}$*	$-1.41_{0.56}$*	$0.66_{0.76}$	$0.10_{1.30}$	$-0.12_{3.07}$	3
CLA index	$1.41_{0.53}$*	$-0.01_{0.54}$	$3.46_{0.87}$*	$-1.09_{0.58}$	$0.26_{0.82}$	$-1.41_{1.39}$	$-0.74_{3.13}$	3

* Significantly segregating QTL ($P \leq 0.05$, calculated by a *t*-test) are marked with an asterisk.
Source: Schennink *et al.* (2009b).

to the QTL for the other traits. Families 5 and 6 did not segregate for any of the QTL on BTA14. The differences in fat percentage between the two daughter groups inheriting alternative sire alleles were 0.53, 0.69, 0.70, 0.68 and 0.93 w/w% in families 1, 2, 3, 4 and 7, respectively. The differences in C18:1 *cis*-9 between the two daughter groups were 1.10, 0.60, 1.37, 1.18 and 1.83 w/w% in families 1, 2, 3, 4 and 7, respectively. This QTL explained 19% of the phenotypic variance for fat percentage, and 10% for C18:1 *cis*-9.

8.6   Exploiting variation in fatty acid composition

Human trial studies show that not all saturated fatty acids affect cholesterol concentrations to the same extent and that some are more unfavourable than others (Mensink *et al.*, 2003). The most unfavourable, hypercholesterolemic fatty acid is C16:0. In the following section, we use C16:0 and UFA to illustrate the opportunities for the different alternatives.

8.6.1   On-farm segregation

On-farm milk segregation is one opportunity for farmers to produce value-added products. First, segregation of cows within a herd can be an option to produce different milk types, and this method can be further improved by using genetic selection (Dooley *et al.*, 2005). Second, by using milking parlours or automatic milking systems equipped with new biosensor technologies, the natural variation between cows can be exploited (Demeter *et al.*, 2009). Mid-infrared spectrometry could be an option for measuring milk composition online. In short, the availability of milking systems equipped with novel biosensors would allow the segregation of different types of milk at the farm level on a daily basis. Stoop (2009) looked at opportunities for on-farm milk segregation for C16:0 or UFA proportion in the cows of the Dutch Milk Genomics Initiative. She found that when the 10% of cows with the lowest fraction of C16:0 were selected, they produce on average 2.3 w/w% less C16:0 than the herd average. Likewise, when the 10% of cows with the best performance for UFA are selected, they produce on average 2.0 w/w% more UFA than the herd average.

An alternative for on-farm segregation is the allocation of farms to different dairy product chains. In that case, one can exploit the differences observed between farms in FA composition. In addition, farms could adopt different feeding strategies, which are tailored towards the production of milk with, for example, increased levels of UFA in the milk (Demeter *et al.*, 2009). In addition, the breeding organization could provide information on breeding values of their bulls for FA composition. The breeding organization can provide that information as soon as information on FA composition is collected on offspring of their bulls. This offers farmers the opportunity to exploit the variation between available bulls in breeding value for FA composition. In addition, the breeding organization could change its breeding programme by including FA composition

in its breeding goal, as will be discussed in the next section. By changing the breeding programme, continuous genetic improvement of the population can be realized.

8.6.2   Breeding for improved composition

A breeding scheme aims at genetic improvement in the breeding goal through the selection of sires and dams to produce the next generation. The breeding goal reflects the combination of traits that the breeder aims at improving through selection. The traits included in the breeding goal of dairy cattle are milk production traits, functional traits, health traits and reproduction.

In the past, some studies questioned the need to change the milk-fat composition for two main reasons: (i) because large changes in milk-fat composition would be required to substantially decrease risks to human health (Maijala, 1995); and (ii) because changes that are positive for one product might be detrimental for other products. The latter reason might imply that multiple breeding goals are needed and that the entire production chain would have to be adapted, for example by separately collecting milk for different end uses (Gibson, 1991). For example, the desired characteristics of milk for human consumption are different from that of milk for cheese production. These differences could be translated into different farms producing different types of milk. This involves changes in the entire dairy chain but offers the opportunity to capitalize on genetic variation in composition of milk. A breeding organization could offer bulls with a good breeding value for cheese-making properties to farmers who specialize in the production of milk for cheese production. At the same time, the breeding organization could offer bulls with a good breeding value for milk-fat composition to farmers who specialize in the production of milk for direct consumption.

However, before a breeding programme can focus on milk-fat composition, a number of issues need to be resolved, including measurement of FA composition, payment system and insight into correlated response. Breeding values of bulls for FA composition need to be estimated from information collected on progeny or other relatives. Measuring FA proportions by gas chromatography analysis is too expensive for use in routine analysis, and cheaper methods are needed for large-scale application. The use of infrared spectroscopy for measuring FA seems promising (Soyeurt *et al.*, 2006). Farmers in many countries are currently paid based on fat and protein content of the milk. This payment system tends to increase rather than decrease the content of C14–16, so FA-based payment should be introduced to stimulate a decrease in C14–16 and an increase in UFA. Selection for a trait might have consequences for the health and welfare of the cow. Negative consequences of selection need to be avoided, which means that relationships between FA composition and health and welfare of the cow need to be estimated and used for the selection of bulls to avoid undesired consequences of selection.

Stoop (2009) used deterministic simulation to determine the rate of genetic improvement from progeny testing of 100 offspring for FA composition. She

showed that single-trait selection could lead to an annual genetic improvement of 0.252 w/w% of UFA or a decrease of 0.267 w/w% of C16:0. This illustrates that genetic changes in FA composition can be realized but that 10 years are needed for making a relative change of 10% in UFA. A similar change can be realized in a much shorter time by changing the feed composition. Further research is needed to compare the advantages and disadvantages of feeding and breeding strategies for changing FA composition and how genetics and nutrition interact.

In current animal breeding schemes, prediction of genetic differences between animals is based on phenotypic observations, which depend on genetic and environmental factors. Using SNP, a number of QTL have been identified that contribute to FA composition. Using this information in breeding schemes could increase the accuracy of selection and the rate of genetic improvement (Kashi *et al.*, 1990; Meuwissen and Van Arendonk, 1992).

8.7   Conclusions

In this chapter, we have given an overview of recent research on genetic variation in milk-fat composition. Recent studies have shown that genetic variation between cows contributes to variation in milk-fat composition. A number of genes and QTL have been identified that contribute to the genetic variation. This is an important step towards a better understanding of the regulation of lipogenesis and will assist us in designing breeding programmes that are tailored towards the production of milk that meets the needs of consumers.

8.8   References

ABE, T., J. SABURI, H. HASEBE, T. NAKAGAWA, T. KAWAMURA, K. SAITO, T. NADE, S. MISUMI, T. OKUMURA, K. KUCHIDA, T. HAYASHI, S. NAKANE, T. MITSUHASI, K. NIRASAWA, Y. SUGIMOTO and E. KOBAYASHI. 2008. Bovine QTL analysis for growth, carcass, and meat quality traits in an F2 population from a cross between Japanese Black and Limousin. *J. Anim. Sci.* 86: 2821–2832.

ALEXANDER, L. J., M. D. MACNEIL, T. W. GEARY, W. M. SNELLING, D. C. RULE and J. A. SCANGA. 2007. Quantitative trait loci with additive effects on palatability and fatty acid composition of meat in a Wagyu–Limousin F2 population. *Anim. Genet.* 38: 506–513.

ASHWELL, M. S., D. W. HEYEN, J. I. WELLER, T. S. SOENSTEGAARD, C. P. VAN TASSELL and H. A. LEWIN. 2005. Detection of quantitative trait loci influencing conformation traits and calving ease in Holstein–Friesian cattle. *J. Dairy Sci.* 88: 4111–4119.

AULDIST, M. J., B. J. WALSH and N. A. THOMSON. 1998. Seasonal and lactational influences on bovine milk composition in New Zealand. *J. Dairy Res.* 65: 401–411.

BARBER, M. C., R. A. CLEGG, M. T. TRAVERS and R. G. VERNON. 1997. Lipid metabolism in the lactating mammary gland. *Biochim. Biophys. Acta* 1347: 101–126.

BAUMGARD, L. H., B. A. CORL, D. A. DWYER, A. SAEBO and D. E. BAUMAN. 2000. Identification

of the conjugated linoleic acid isomer that inhibits milk fat synthesis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 278: R179–R184.

BITMAN, J., D. L. WOOD, R. H. MILLER, J. C. WILK and E. D. MOORE. 1995. Comparison of lipid composition of milk from Half-Danish Jersey cows and United States Jersey cows. *J. Dairy Sci.* 78: 655–658.

BOBE, G., D. C. BEITZ, A. E. FREEMAN and G. L. LINDBERG. 1999. Associations among individual proteins and fatty acids in bovine milk as determined by correlations and factor analysis. *J. Dairy Sci.* 66: 523–536.

BOICHARD, D., C. GROHS, F. BOURGEOIS, F. CERQUEIRA, R. FAUGERAS, A. NEAU, R. RUPP, Y. AMIGUES, M. Y. BOSCHER and H. LEVEZIEL. 2003. Detection of genes influencing economic traits in three French dairy cattle breeds. *Genet. Sel. Evol.* 35: 77–101.

CASES, S., S. J. SMITH, Y. W. ZHENG, H. M. MYERS, S. R. LEAR, E. SANDE, S. NOVAK, C. COLLINS, C. B. WELCH, A. J. LUSIS, S. K. ERICKSON and R. V. FARESE JR. 1998. Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proc. Natl. Acad. Sci. USA* 95: 13018–13023.

CHEN, H. C., S. J. SMITH, Z. LADHA, D. R. JENSEN, L. D. FERREIRA, L. K. PULAWA, J. G. MCGUIRE, R. E. PITAS, R. H. ECKEL and R. V. FARESE JR. 2002. Increased insulin and leptin sensitivity in mice lacking acyl CoA:diacylglycerol acyltransferase 1. *J. Clin. Invest.* 109: 1049–1055.

CHILLIARD, Y., A. FERLAY and M. DOREAU. 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livest. Prod. Sci.* 70: 31–48.

CHUNG, M., S. HA, S. JEONG, J. BOK, K. CHO, M. BAIK and Y. CHOI. 2000. Cloning and characterization of bovine stearoyl CoA desaturase1 cDNA from adipose tissues. *Biosci. Biotechnol. Biochem.* 64: 1526–1530.

CLOP, A., C. OVILO, M. PEREZ-ENCISO, A. CERCOS, A. TOMAS, A. FERNANDEZ, A. COLL, J. M. FOLCH, C. BARRAGAN, I. DIAZ, M. A. OLIVER, L. VARONA, L. SILIO, A. SANCHEZ and J. L. NOGUERA. 2003. Detection of QTL affecting fatty acid composition in the pig. *Mammal. Genome* 14: 650–656.

COLLOMB, M., W. BISIG, U. BÜTIKOFER, R. SIEBER, M. BREGY and L. ETTER. 2008. Fatty acid composition of mountain milk from Switzerland: Comparison of organic and integrated farming systems. *Int. Dairy J.* 18: 976–982.

DEMETER R. M., M. P. M. MEUWISSEN, A. G. J. M. OUDE LANSINK and J. A. M. VAN ARENDONK. 2009. Scenarios for a future dairy chain in the Netherlands. *Neth. J. Agric. Sci.* 56: 301–323.

DOOLEY, A.E., W. J. PARKER, H. T. BLAIR and E. M. HURLEY. 2005. Implications of on-farm segregation for valuable milk characteristics. *Agric. Syst.* 85: 82–97.

ELGERSMA, A., S. TAMMINGA and G. ELLEN. 2006. Modifying milk composition through forage. *Anim. Feed Sci. Technol.* 131: 207–225.

FAMULA, T. R., J. F. MEDRANO, E. J. DEPETERS and S. L. BERRY. 1995. Estimation of heritability and genetic correlations among fatty acid components of milk in Holstein cows. *J. Dairy Sci.* 78 (suppl.1): 194 (Abstr.).

GEORGES, M., D. NIELSEN, M. MACKINNON, A. MISHRA, R. OKIMOTO, A. T. PASQUINO, L. S. SARGEANT, A. SORENSEN, M. R. STEELE, X. ZHAO, J. E. WOMACK and I. HOESCHELE. 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. *Genetics*: 907–920.

GERMAN, J. B. and C. J. DILLARD. 2006. Composition, structure and absorption of milk lipids: A source of energy, fat-soluble nutrients and bio-active molecules. *Crit.*

Rev. Food Sci. Nutr. 46: 57–92.

GIBSON, J. P. 1991. The potential for genetic change in milk-fat composition. *J. Dairy Sci.* 74: 3258–3266.

GRISART, B., W. COPPIETERS, F. FARNIR, L. KARIM, C. FORD, P. BERZI, N. CAMBISANO, M. MNI, S. REID, P. SIMON, R. SPELMAN, M. GEORGES and R. SNELL. 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Res.* 12: 222–231.

HECK, J.M.L., H.J.F. VAN VALENBERG, J. DIJKSTRA and A.C.M. VAN HOOIJDONK. 2009. Seasonal variation in the Dutch bovine raw milk composition. *J. Dairy Sci.* 92: 4745–4755.

HU, F. B. and W. C. WILLETT. 2002. Optimal diets for prevention of coronary heart disease. *JAMA* 288: 2569–2578.

HULSHOF K. F., M. A. VAN ERP-BAART, M. ANTTOLAINEN, W. BECKER, S. M. CHURCH, C. COUET, E. HERMANN-KUNZ, H. KESTELOOT. T. LETH, I. MARTINS, O. MOREISAS, J. M. AMELSVOORT, A. ARO, A. G. KATAFOS. D. LANZMANN-PETITHORY and G. VAN POPPEL. 1999. Intake of fatty acids in western Europe with emphasis on trans fatty acids: the TRANSFAIR study. *Eur. J. Clin. Nutr.* 53: 143–157.

ISO-IDF. 2002a. Milk fat – Determination of the fatty acid composition by gas–liquid chromatography. ISO 15885-IDF 184. International Dairy Federation, Brussels.

ISO-IDF. 2002b. Milk fat – Preparation of fatty acid methyl esters. ISO 15884-IDF 182. International Dairy Federation, Brussels.

JENSEN, R. G. 2002. Invited review: The composition of bovine milk lipids: January 1995 to December 2000. *J. Dairy Sci.* 85: 295–350.

KARAMICHOU, E., R. I. RICHARDSON, G. R. NUTE, K. P. GIBSON and S. C. BISHOP. 2006. Genetic analyses and quantitative trait loci detection, using a partial genome scan, for intramuscular fatty acid composition in Scottish Blackface sheep. *J. Anim. Sci.* 84: 3228–3238.

KARIJORD, Ø., N. STANDAL and O. SYRSTAD. 1982. Sources of variation in composition of milk fat. *Z. Tierzüchtg. Züchtgsbiol.* 99: 81–93.

KASHI, Y., E. HALLERMAN and M. SOLLER. 1990. Marker assisted selection of candidate bulls for progeny testing programmes. *Anim. Rod.* 51: 63.

KELSEY, J. A., B. A. CORL, R. J. COLLIER and D. E. BAUMAN. 2003. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. *J. Dairy Sci.* 86: 2588–2597.

LAWLESS, F., C. STANTON, P. L'ESCOP, R. DEVERY, P. DILLON and J. J. MURPHY. 1999. Influence of breed on bovine milk *cis*-9, *trans*-11-conjugated linoleic acid content. *Livest. Prod. Sci.* 62: 43–49.

LENGI, A. J. and B. A. CORL. 2007. Identification and characterization of a novel bovine stearoyl-CoA desaturase isoform with homology to human SCD5. *Lipids* 42: 499–508.

LOCK, A. L. and P. C. GARNSWORTHY. 2002. Independent effects of dietary linoleic and linolenic fatty acids on the conjugated linoleic acid content of cows' milk. *Anim. Sci.* 74: 163–176.

MACGIBBON, A. K. H. and M. W. TAYLOR. 2006. Composition and structure of bovine milk lipids. In: *Advanced Dairy Chemistry. Vol. 2: Lipids*, 3rd edition, edited by P. F. Fox and P. L. H. McSweeney, Springer, New York, pp. 1–42.

MAIJALA, K. 1995. Current possibilities of breeding to influence milk composition from the viewpoint of human nutrition and health. In: *Proc. NJF/NMR Seminar no. 252. Milk in Nutrition Effects of Production and Processing Factors*, Turku, Finland, p. 23.

MELE, M., G. CONTE, B. CASTIGLIONI, S. CHESSA, N. P. MACCIOTTA, A. SERRA, A. BUCCIONI, G. PAGNACCO and P. SECCHIARI. 2007. Stearoyl-coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. *J. Dairy Sci.* 90: 4458–4465.

MENSINK, R. P., P. L ZOCK, A. D. M. KESTER and M. B. KATAN. 2003. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am. J. Clin. Nutr.* 77: 1146–1155.

MEUWISSEN, T. H. E. and J. A. M. VAN ARENDONK. 1992. Potential improvements in rate of genetic gain from marker-assisted selection in dairy cattle breeding schemes. *J. Dairy Sci.* 75: 1651–1659.

MOIOLI, B., G. CONTARINI, A. AVALLI, G. CATILLO, L. ORRU, G. DE MATTEIS, G. MASOERO and F. NAPOLITANO. 2007. Short communication: Effect of stearoyl-coenzyme A desaturase polymorphism on fatty acid composition of milk. *J. Dairy Sci.* 90: 3553–3558.

MORRIS, C. A., N. G. CULLEN, B. C. GLASS, D. L. HYNDMAN, T. R. MANLEY, S. M. HICKEY, J. C. MCEWAN, W. S. PITCHFORD, C. D. BOTTEMA and M. A. LEE. 2007. Fatty acid synthase effects on bovine adipose fat and milk fat. *Mammal. Genome* 18: 64–74.

NRS. 2006. *Year Statistics 2005*, NRS, Arnhem, the Netherlands.

PALMQUIST, D. L. 2006. Milk fat: Origin of fatty acids and influence of nutritional factors thereon. In: *Advanced Dairy Chemistry. Vol. 2: Lipids*, 3rd edition, edited by P. F. Fox and P. L. H. McSweeney, Springer, New York, pp. 43–92.

PALMQUIST, D. L. and A. D. BEAULIEU. 1993. Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* 76: 1753–1771.

PEREIRA, S. L., A. E. LEONARD and P. MUKERJI. 2003. Recent advances in the study of fatty acid desaturases from animals and lower eukaryotes. *Prostaglandins Leukot. Essent. Fatty Acids* 68: 97–106.

PERFIELD, J. W., II, P. DELMONTE, A. L. LOCK, M. P. YURAWECZ and D. E. BAUMAN. 2006. *Trans*-10, *trans*-12 conjugated linoleic acid does not affect milk fat yield but reduces delta9-desaturase index in dairy cows. *J. Dairy Sci.* 89: 2559–2566.

PERFIELD, J. W., II, A. L. LOCK, J. M. GRIINARI, A. SAEBO, P. DELMONTE, D. A. DWYER and D. E. BAUMAN. 2007. *Trans*-9, *cis*-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows. *J. Dairy Sci.* 90: 2211–2218.

PRECHT, D. and J. MOLKENTIN. 1999. Analysis and seasonal variation of conjugated linoleic acid and further *cis-/trans*-isomers of C18:1 and C18:2 in bovine milk fat. *Kieler Milchwirtschaftliche Forschungsberichte* 51: 63–78.

RENNER, E. and U. KOSMACK. 1974a. Genetic aspects concerning the composition of fatty acids in milk-fat: 1. Delimitation to non-genetic factors. *Züchtungskunde* 46: 90–99.

RENNER E. and U. KOSMACK. 1974b. Genetic aspects of fatty acid composition of milk fat: 2. Fatty acid pattern of milk from progeny groups. *Züchtungskunde* 46: 217–26.

ROYAL, M. D. and P. C. GARNSWORTHY. 2005. Estimation of genetic variation in Δ9-desaturase enzyme activity in dairy cows. In: *Proc. Br. Soc. Anim. Sci.*, York, UK, British Society of Animal Science, Penicuik, UK, p. 52.

SANCHEZ M. P., N. IANNUCCELLI, B. BASSO, J. P. BIDANEL, Y. BILLON, G. GANDEMER. H. GILBERT, C. LARZUL, C. LEGAULT, J. RIQUET, D. MILAN and P. LE ROY. 2007. Identification of QTL with effects on intramuscular fat content and fatty acid composition in a Duroc × Large White cross. *BMC Genetics* 8: 55.

SCHENNINK, A., W. M. STOOP, M. H. P. W. VISKER, J. M. L. HECK, H. BOVENHUIS, J. J. VAN DER POEL, H. J. F. VAN VALENBERG and J. A. M. VAN ARENDONK. 2007. DGAT1 underlies

large genetic variation in milk-fat composition of dairy cows. *Anim. Genet.* 38: 467–473.

SCHENNINK, A., J. M. L. HECK, H. BOVENHUIS, M. H. P. W. VISKER, H. J. F. VAN VALENBERG and J. A. M. VAN ARENDONK. 2008. Milk fatty acid unsaturation: Genetic parameters and effects of stearoyl-CoA desaturase (SCD1) and acyl CoA:diacylglycerol acyltransferase 1 (DGAT1). *J. Dairy Sci.* 91: 2135–2143.

SCHENNINK A., H. BOVENHUIS, K. M. LÉON-KLOOSTERZIEL, J. A. M. VAN ARENDONK and M. H. P. W. VISKER. 2009a. Effects of polymorphisms in the FASN, OLR1, PPARGC1A, PRL, and STAT5A genes on bovine milk-fat composition. *Anim. Genet.* doi:10.1111/j.1365-2052.2009.01940.x

SCHENNINK, A., W. M. STOOP, M. H. P. W. VISKER, J. J. VAN DER POEL, H. BOVENHUIS and J. A. M. VAN ARENDONK. 2009b. Genome-wide scan for bovine milk-fat composition II. QTL for long chain fatty acids. *J. Dairy Sci.* 92: 4676–4682.

SCHNABEL, R. D., T. S. SONSTEGARD, J. F. TAYLOR and M. S. ASHWELL. 2005. Whole genome scan to detect QTL for milk production, conformation, fertility and functional traits in two US Holstein families. *Anim. Genet.* 36: 408–416.

SCHROOTEN C., H. BOVENHUIS, W. COPPIETERS and J. A. M. VAN ARENDONK. 2000. Whole genome scan to detect quantitative trait loci for conformation and functional traits in dairy cattle. *J. Dairy Sci.* 83: 795–806.

SHORTEN, P. R., T. B. PLEASANTS and G. C. UPRETI. 2004. A mathematical model for mammary fatty acid synthesis and triglyceride assembly: The role of stearoyl CoA desaturase (SCD). *J. Dairy Res.* 71: 385–97.

SMITH, S. J., S. CASES, D. R. JENSEN, H. C. CHEN, E. SANDE, B. TOW, D. A. SANAN, J. RABER, R. H. ECKEL and R. V. FARESE JR. 2000. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking DGAT. *Nat. Genet.* 25: 87–90.

SOYEURT, H., P. DARDENNE, A. GILLON, C. CROQUET, S. VANDERICK, P. MAYERES, C. BERTOZZI and N. GENGLER. 2006. Variation in fatty acid contents of milk and milk-fat within and across breeds. *J. Dairy Sci.* 89: 4858–4865.

SPELMAN, R. J., D. J. GARRICK and J. A. M. VAN ARENDONK. 1999. Utilisation of genetic variation by marker assisted selection in commercial dairy cattle populations. *Livest. Prod. Sci.* 59: 51–60.

SPELMAN, R. J., C. A. FORD, P. MCELHINNEY, G. C. GREGORY and R. G. SNELL. 2002. Characterization of the DGAT1 gene in the New Zealand dairy population. *J. Dairy Sci.* 85: 3514–3517.

STOOP, W. M. 2009. Genetic variation in bovine milk fat composition. PhD thesis, Wageningen University, the Netherlands, ISBN 978-90-8585-355-8, 176 pp.

STOOP, W. M., J. A. M. VAN ARENDONK, J. M. L. HECK, H. J. F. VAN VALENBERG and H. BOVENHUIS. 2008. Genetic parameters for major milk fatty acids and milk production traits of Dutch Holstein–Friesians. *J. Dairy Sci.* 91: 385–394.

STOOP, W. M., H. BOVENHUIS, J. M. L. HECK and J. A. M. VAN ARENDONK. 2009a. Effect of lactation and energy status on milk fat composition of Holstein–Friesian cows. *J. Dairy Sci.* 92: 1469–1478.

STOOP, W. M., A. SCHENNINK, M. H. P. W. VISKER, E. MULLAART, J. A. M. VAN ARENDONK and H. BOVENHUIS. 2009b. Genome-wide scan for bovine milk-fat composition. I. Quantitative trait loci for short and medium chain fatty acids. *J. Dairy Sci.* 92: 4664– 4675.

STOOP, W. M., H. BOVENHUIS and J. A. M. VAN ARENDONK. 2009c. Genetic correlations between winter and summer milk fat composition. *J. Dairy Sci.* (submitted).

STULL, J. W. and W. H BROWN. 1964. Fatty acid composition of milk: 2. Some differences in

common dairy breeds. *J. Dairy Sci.* 47: 1412.

TANIGUCHI, M., T. UTSUGI, K. OYAMA, H. MANNEN, M. KOBAYASHI, Y. TANABE, A. OGINO and S. TSUJI. 2004. Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black cattle. *Mammal Genome* 15: 142–148.

THALLER, G., W. KRAMER, A. WINTER, B. KAUPE, G. ERHARDT and R. FRIES. 2003. Effects of DGAT1 variants on milk production traits in German cattle breeds. *J. Anim. Sci.* 81: 1911–1918.

WINTER, A., W. KRAMER, F. A. WERNER, S. KOLLERS, S. KATA, G. DURSTEWITZ, J. BUITKAMP, J. E. WOMACK, G. THALLER and R. FRIES. 2002. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA:diacylglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content. *Proc. Natl Acad. Sci. USA* 99: 9300–9305.

9

Cows' diet and milk composition

O. M. Harstad, Norwegian University of Life Sciences, Norway and H. Steinshamn, Norwegian Institute for Agricultural and Environmental Research, Norway

Abstract: The effect of cows' diets on milk composition is discussed, focusing on those components of particular interest for human health. First dietary sources of fatty acids and their digestion and metabolism are reviewed. Then feeding factors affecting milk fat content and fatty acid composition are discussed, with emphasis on those factors related to forage and fat supplements. The effects of diet on protein content and composition and milk content of minerals and vitamins are reviewed. Feeding strategies for improving the nutritional quality of milk are summarized.

Key words: milk nutritional quality, fatty acid composition, effects of diet, forage source, fat supplements, vitamins, minerals.

9.1 Introduction: cows' diet and milk composition

In ruminants, ingested feed is exposed to fermentation in the forestomach, a process that makes it more complicated to manipulate the composition of their products than in monogastric animals. However, cows' milk composition can be altered by the feeding regime, but the extent varies greatly among milk components. The main aim of this chapter is to discuss effects of diet on milk composition with special emphasis on those components which are modifiable and that are important from a human health perspective.

9.2   Diet and nutritional quality of milk

The term 'nutritional quality' refers to several components in milk and milk products which are of great importance in human nutrition. Most attention has been paid to the fat fraction, probably due to its health effects and good prospects of being manipulated by feeding. Oleic acid (c9-18:1), conjugated linoleic acid (c9t11-18:2, CLA), n-3 fatty acids (FA) and some short- and medium-chain FA are considered to promote positive health effects, whereas negative health effects are attributed in particular to the high fraction of lauric acid (12:0), myristic acid (14:0) and stearic acid (16:0) (Dewhurst *et al.*, 2006; Chilliard *et al.*, 2007; Haug *et al.*, 2007). Milk is also a valuable source of essential amino acids, and contains in addition a wide array of bioactive proteins which are considered to have positive health effects (Haug *et al.*, 2007). Moreover, milk is a good source of some minerals and vitamins, which are also considered to have positive health effects (Haug *et al.*, 2007). Besides feeding and management, milk nutritional quality is also influenced by breeding and stage of lactation (Palmquist *et al.*, 1993). This chapter is restricted to discussing the prospects of influencing milk nutritional quality by feeding.

Fatty acids in milk originate from the feed and FA synthesized in the udder (*de novo* synthesized) from acetic and butyric acids which are end-products from the fermentation in the rumen. Both the mammary supply of individual FA and of the substrates for the *de novo* synthesis of FA varies, depending on feeding. Therefore, fat content as well as its composition may be substantially altered by nutrition.

Milk protein content is most affected by mammary supply of energy and amino acids (AA). Under practical conditions, there are, however, rather limited possibilities to manipulate mammary supply of AA and energy for synthetic processes. Moreover, proteins have a genetically determined AA sequence. As a consequence, influencing milk protein content as well as its composition by feeding is limited.

The contents of some fat-soluble vitamins are also easily manipulated because their contents vary among feeds, they are transformed to milk and they are not synthesized endogenously. In contrast, the milk content of B-vitamins is only marginally influenced by feeding due to the mechanism of adapting their microbial synthesis to feed supply (McDonald *et al.*, 2002).

All minerals required for producing milk have to be ingested with the feeds and/or supplements because none of them is synthesized by the animal. Some minerals are a component of other chemical structures, and their content depends on the variability of that particular component. Other minerals simply diffuse from blood to milk, and the resultant concentration in milk is directly related to feed concentration and the extent of their transfer from blood to milk.

Manipulating milk composition assumes that the diet also can be altered. However, there are restrictions in practice because a lot of other aspects also have to be taken into account. In late lactation with a rather low milk yield, roughage may cover the cows' requirements of energy and specific nutrients.

Under such conditions, milk composition reflects roughage quality. High quality pasture is rich in both energy and protein, and daily milk yield may be maintained at 30 kg or more with no or only small amounts of supplemental feeds. Accordingly, milk composition reflects the quality and characteristics of the pasture. However, with the exception of cows in late lactation and cows grazing on high quality pasture, supplemental feeds are necessary under most feeding situations. The main objective of using supplementary feeds is to complement the basal diet of roughage. However, the composition of the supplementary feeds may have a decisive effect on the milk's nutritional quality. Use of properly composed supplementary feeds is a convenient way to improve the milk's nutritional quality in practice. The challenge is to optimize the supplemental feeds with respect to their effect on milk quality in a profitable way.

9.3   Milk fat content and composition

Cow's milk contains 3.5 to 5% fat, and of the fat fraction a mixture of triacylglycerols represents as much as 95–96% (Table 9.1). Phospholipids associated with the fat globule membrane constitute about 1%, and the remaining fractions are fat components like diacylglycerol, cholesterol and free FA.

9.3.1   Dietary sources of fatty acids

The fat content and FA composition vary widely among feedstuffs. Triacylglycerides are the major lipid type in cereal grains, oilseeds and animal fats, whereas glycolipids and phospholipids constitute the major part of the fat fraction in forages (Hawke, 1973). The sources of fatty acids in cows' diets may be divided into two broad categories: forages and concentrates.

Forages
Forages have a high content of cellulolytic material, and cattle have evolved to maximize the utilization of feedstuffs that are without alternative nutritive value. Forages contribute to the milk FA in two ways. First, the rumen microorganisms ferment cell walls in the forages to acetate and butyrate, which are the precursors

Table 9.1   Composition of bovine milk lipids

Class of lipid	Percentage of total milk lipids
Triglycerides of fatty acids	95–96
Diglycerides	1.26–1.59
Free fatty acids	0.10–0.44
Phospholipids (total)	0.80–1.00
Sterols	0.22–0.41

Source: Kurtz (1978).

for *de novo* synthesis of milk FA in the mammary gland. Secondly, forages also contribute FA directly. Fats from forages are the only fat contributor in situations without supplemental feeds. Even in early lactation with supplemental feeds, fats from roughages account for 20–40% of total FA intake. Forage lipid content in grazed and harvested plant parts ranges from 30 to 100 g/kg DM (Hawke, 1973). More than 95% of total FA are α-linolenic (18:3*n*-3), linoleic (18:2*n*-6) and palmitic (16:0) acids, and α-linolenic acid represents more than 40% of total FA (Table 9.2).

The lipid content and its FA composition depend on several factors such as plant species, growth stage, temperature, light intensity and management (Hawke, 1973; Dewhurst and King, 1998; Dewhurst *et al.*, 2001; Boufaied *et al.*, 2003a). FA concentrations are lower during midsummer than in spring and late summer (Bauchart *et al.*, 1984), the concentration declines with advancing maturity (spring growth) (Boufaied *et al.*, 2003a), and frequent cuts that keep the plants in a young stage of development maintain high FA concentrations (Dewhurst *et al.*, 2001; Boufaied *et al.*, 2003a). The high leaf/stem ratio achieved with short regrowth periods (frequent cuts or grazing) or with use of N fertilizer increases the total content of FA, particularly the concentration of 18:3*n*-3 (Boufaied *et al.*, 2003a; Elgersma *et al.*, 2005). Another major factor affecting forage FA content and composition is oxidative loss of polyunsaturated FA (PUFA), particular 18:3*n*-3, during wilting prior to ensiling and haymaking (Dewhurst and King, 1998; Boufaied *et al.*, 2003a; Elgersma *et al.*, 2003). FA in fresh forage is to a large extent esterified in major classes of lipids (galacto- and phospholipids). During ensiling the plant lipids undergo extensive hydrolysis (lipolysis), which leads to increased levels of free FA (Elgersma *et al.*, 2003; Vanhatalo *et al.*, 2007; Van Ranst *et al.*, 2009). However, lipolysis during ensiling has only a small effect on the composition and total amount of FA in the silage, provided that the anaerobic conditions required for good ensiling are present (Dewhurst and King, 1998; Van Ranst *et al.*, 2009; Arvidsson *et al.* 2009). Formic acid additives have been found to increase both the total content and 18:3*n*-3 FA in the silage when compared with no additives or inoculants, probably due to effluent loss (Dewhurst and King, 1998; Boufaied *et al.*, 2003b; Shingfield *et al.*, 2005).

Concentrates

Usually, the term 'concentrates' refers to feeds with high feeding value in terms of energy and/or protein. They comprise a wide range of different feedstuffs, including cereal grains and oilseeds. The lipid content of cereal grains varies with species, but is in the range 1–6% on a DM basis (Table 9.2). Despite their relatively low fat content, cereal grains may account for a significant proportion of total FA intake, especially in early lactation when cereal grains usually constitute a major part of the concentrate. Cereal fats are composed mainly of unsaturated FA, but their proportion varies widely among species (Table 9.2). Oats differ from barley, with a higher fat content, and a fat fraction having a higher proportion of *c*9-18:1 at the expense of *c*9*c*12-18:2.

Table 9.2 Fat content and composition (g/100 g fatty acids) of various forages and concentrates

	Fat	12:0	14:0	16:0	16:1	18:0	18:1	18:2*n*-6	18:3*n*-3
Forages (g fatty acids per kg DM):[a]									
Timothy (early heading)	19.82	0.4	0.7	17.7	2.3	1.9	5.5	19.4	52.1
Timothy (early flowering)	16.80	0.4	1.0	18.9	2.1	2.0	6.5	22.1	47.0
Annual rygrass (cv. 'Aubabe')	26.50	0.2	0.5	17.5	2.6	1.5	5.1	15.1	57.4
White clover (cv. 'Merit')	28.13	0.2	0.4	16.9	2.7	2.9	5.1	16.5	55.3
Red clover (cv. 'Walter')	21.56	0.3	0.5	18.8	2.6	3.8	8.0	22.9	43.0
Alfalfa (cv. '120')	17.20	0.5	0.8	24.7	2.5	4.5	5.1	21.8	40.2
Concentrates (g fat per kg DM):									
Oats[b]	55.70	0.9	1.7	16.6	0.6	1.4	33.9	38.3	1.4
Barley[b]	22.40	–	0.4	22.3	–	1.4	13.4	53.9	5.1
Rapeseed[b]	420.80	–	–	4.0	0.2	1.6	58.1	21.2	11.8
Soybean oil[b]		–	0.1	10.7	0.1	3.8	22.0	52.8	9.1
Sunflower oil[c]		0.5	0.2	6.8	0.1	4.7	18.6	68.2	0.5
Linseed oil[c]		–	–	4.8	–	4.7	19.9	15.9	52.7

Sources: [a]Recalculated from Boufaied *et al.* (2003a,b); [b]Norwegian Feed Table (2008); [c]White (2000).

Oilseeds are, by definition, seeds from plants grown mainly for their high fat content. Common species are soybean, rape, sunflower, safflower and linseed. Both the oil and the raw seeds can be mixed into the diet of ruminants. However, oilseeds are normally included in the diets as a protein source. Therefore, the fat-extraction residues are most commonly used. The content of fat in the residues depends upon the extraction process employed and its efficiency, but most products have a fat content ranging between 1 and 10% on DM basis. Because of its relatively low fat content and low inclusion in most diets for dairy cows, oilseed meal accounts for only a relatively small proportion of total FA intake. The typical FA composition of oilseed meal is shown in Table 9.2. Almost 70% of the FA in sunflower oil is 18:2n-6 whilst the content of 18:3n-3 is negligible, whereas linseed oil contains approximately 50% 18:3n-3 and only 15% 18:2n-6. Rapeseed oil typically contains almost 60% 18:1.

9.3.2   Digestion and metabolism of dietary fat

The fat composition of cows' milk differs substantially from that in the diet. This is due to the extensive restructuring of the feed fat fraction during its digestion and metabolism and *de novo* synthesis of short- and medium-chain fatty acids in the mammary gland. The two main restructuring processes involved are: (a) biohydrogenation of unsaturated fatty acids in the rumen, and (b) desaturation of fatty acids in the mammary gland.

Fat metabolism in the rumen
The diet of dairy cows contains predominantly PUFA. The processes in the rumen are (a) hydrolysis of dietary lipids, (b) hydrogenation of unsaturated FA, and (c) synthesis of microbial fat.

Hydrolysis of dietary lipids
Microbes in the rumen release enzymes which split off the fatty acids (and sugars from glycolipids) from the glycerol backbone (Harfoot and Hazlewood, 1997). The glycerol and the sugar are fermented to volatile fatty acids (VFA). Rumen lipolysis generally occurs quite rapidly, but is slowed down by low ruminal pH (Harfoot and Hazlewood, 1997). The FA released in the rumen pass to the abomasum and then to the small intestine, where they are absorbed. However, the FA profile that reaches the intestine is very different from the fat ingested because of the extensive biohydrogenation that occurs in the rumen.

Biohydrogenation in the rumen
Free unsaturated FA are quickly hydrogenated to saturated FA. Between 75% and 90% of 18:2n-6 and 85-95% of 18:3n-3 acids are biohydrogenated in the rumen (Doreau and Ferlay, 1994) to the corresponding saturated FA (18:0) that flow to the small intestine. The extent of biohydrogenation decreases when feeding diets that result in low ruminal pH. This is probably due to the negative influence of low pH on the cellulolytic bacteria, which are considered to be the

main biohydrogenators (Harfoot and Hazlewood, 1997). During the biohydro-genation process, intermediates escape from the rumen and are incorporated into milk fat. This accounts for the presence of different trans-fatty acids like *c9t*11-18:2 (CLA) and *t*11-C18:1 (vaccenic acid) in milk fat. Under low rumen pH conditions, a different set of *trans*-intermediates may be produced. One of these intermediates, *t*10*c*12-18:2, has an inhibitory effect on milk fat synthesis, and milk fat depression may result (Griinari and Bauman, 2006).

Microbial fat

Rumen microorganisms account for 10–20% of the total lipid present in the rumen (Harfoot and Hazlewood, 1997). These organisms incorporate hydrogenated FA into their lipid, which explains why saturated FA constitute as much as approximately 70% of the microbial fat fraction (Harfoot and Hazlewood, 1997). A characteristic of bovine milk fat is the presence of FA with a branched carbon chain or with an odd number of carbon atoms. These acids are primarily produced by microorganisms in the digestive tract from branched amino acids and from propionate. Because propionate has three carbon atoms, synthesis of FA in which propionate is the precursor gives rise to FAs with an uneven number of carbon atoms.

Fat digestion in the small intestine and transport to the udder

Saturated free FA (SFA) dominate the lipid fraction that flows out of the rumen. About two-thirds of SFA are 18:0 and about one-third 16:0 (Harfoot and Hazlewood, 1997). In the rumen, with a pH near neutral, most of the free FA liberated during lipolysis associate as potassium, sodium or calcium salts. The free FA released adsorb to the surface of small feed particles that flow out of the rumen as a part of the digesta. In the abomasum with acidic conditions (pH 2.0), the fatty acid salts are dissociated. In the intestine, a complex called a micelle is formed to promote the absorption of the FA. In the intestinal cells, the FA combines with glycerol, and triacylglycerides are rebuilt. With the purpose of being soluble and transportable in blood, the triacylglycerides are packaged into two types of lipoprotein particles; chylomicrons and very low density lipo-proteins (VLDL). The lipoproteins are hydrolysed by lipoprotein lipase (LPL) at the basal mammary membrane and the released FA are taken up by the gland.

Milk fat synthesis

Milk fat synthesis in the udder can be divided into four steps: (a) formation of acetyl-CoA; (b) elongation of the carbon chain; (c) formation of double bonds (desaturation); and (d) formation of triacylglycerides.

 The first step in milk fat synthesis is formation of malonyl-CoA by binding of HCO_3^- to acetyl CoA. Malonyl-CoA is the initial substrate for formation of the fatty acid chain, and is the acceptor for the next C_2 unit (Acetyl-CoA) (Sjaastad *et al.*, 2003). In the ruminant animal, acetate is the major source of acetyl-CoA. The FA chains are elongated by successive additions of acetyl-CoA up to 16 carbon atoms. The mammary epithelial cells contain a unique enzyme system

that releases the FA at different stages in the synthesis. Bovine milk fat will thus contain saturated FA with 4, 6, 8, 10, 12, 14 and 16 carbon atoms. The *de novo* synthesized FA constitute about half of the FA, and the other half is absorbed from the blood (Chilliard *et al.*, 2000). Palmitic acid comes from both *de novo* synthesis (50%) and from circulating blood.

In the udder, desaturation of FA such as 12:0, 14:0, 16:0, 18:0 and *t*11-18:1 takes place, the products being 12:1, 14:1, 16:1, *c*9-18:1 and *c*9*t*11-18:2, respectively. The preferred substrate for the acting enzyme, Δ-9-desaturase, is 18:0. About 40% of the absorbed 18:0 is desaturated to *c*9-18:1 and of *t*11-18:1 to *c*9*t*11-18:2 (Griinari *et al.*, 2000). This explains why bovine milk is a good source of *c*9-18:1 and *c*9*t*11-18:2, respectively. The udder cannot make double bonds closer than carbon atom 9 from the terminal methyl group. Consequently, the milk content of *c*9*c*12-18:2 and *c*9*c*12*c*15-18:3 depends on the supply of these to the udder.

The FA *de novo* synthesized, FA absorbed, and desaturated FA in the mammary gland are finally esterified to glycerol, forming triacylglycerols, which are secreted as milk fat globules. Thus, the fat fraction in bovine milk contains a wide range of saturated and unsaturated fatty acids, and is characterized by (Table 9.3):

- A high proportion of saturated fatty acids where palmitic acid (16:0) predominates
- Containing low and medium chain length FA (4 to 14)
- Low proportions of unsaturated FA with exception of oleic acid (*c*9-18:1)
- Containing conjugated and *trans* fatty acids.

Table 9.3   Normal content of major fatty acids of bovine milk

Fatty acids (common name)	Average range (wt%)
Saturated fatty acids:	
4:0 (butyric)	2–5
6:0 (caproic)	1–5
8:0 (caprylic)	1–3
10:0 (capric)	2–4
12:0 (lauric)	2–5
14:0 (myristic)	8–14
15:0 (pentadecanoic)	1–2
16:0 (palmitic)	22–35
17:0 (margaric)	0.5–1.5
18:0 (stearic)	9–14
Unsaturated fatty acids:	
16:1 (palmitoleic)	1–3
18:1 (*c*9 oleic acid)	20–30
18:2 (*c*9*c*12 linoleic acid)	1–3
18:3 (*c*9*c*12*c*15 linolenic acid)	0.5–2

Source: Kaylegian and Lindsay (1995).

Table 9.4   Effects of forage source on milk FA profile of cow milk fat

	Concentrate-based diet	Maize silage	Ryegrass		Natural grassland	
			Silage	Hay	Hay	Pasture
Forage % of dry matter intake	35	90	87	90	87	97
Milk FA (g/100 g total FA)						
4	4.28	4.46	4.88	4.22	4.54	3.23
6 + 8	4.75	4.35	4.38	4.06	5.75	2.81
10 + 12 + 14	21.86	19.28	18.66	19.55	19.93	14.82
16	33.47	30.96	32.13	30.22	28.59	22.86
18	6.65	7.89	7.93	8.16	8.13	11.40
c9-18:1	14.09	16.66	15.97	15.38	15.97	24.10
t11-18:1	0.62	1.04	0.87	1.83	1.36	3.69
c9c12-18:2	1.77	1.46	1.09	1.00	1.08	1.09
c9c12c15-18:3	0.46	0.24	0.94	1.02	1.25	0.99
c9 t11-18:2 (CLA)	0.39	0.66	0.46	0.87	0.72	1.72

Source: adapted from Ferlay *et al.* (2006).

The milk fat proportion of *trans*-FA and conjugated fatty acids is usually low (each FA contributes usually <1% of total FA) but varies greatly with diet (see Table 9.4). The main isomers are t11-18:1 and c9t11-18:2.

9.3.3   Effects of forage source on milk fat content and composition
Fresh grass and conservation effects on milk FA composition
Grazing increases the milk fat proportion of 18:0, c9-18:1, t1118:1, c9t11-18:2 (CLA) and c9c12c15 18:3n-3 and decreases 10:0-16:0 when compared to winter diets based on conserved forages (Chilliard *et al.*, 2001; Bargo *et al.*, 2006; Ferlay *et al.*, 2006; see Table 9.4). Dhiman *et al.* (1999) observed a linear increase in 18:3n-3, t11- 18:1 and c9t11-18:2 when the proportion of pasture in the diet was increased from a third to 100%. The milk FA composition changes quickly (within a few days) after transition from fresh grass pasture to silage diet and vice versa (Chilliard *et al.*, 2001; Elgersma *et al.*, 2004). This grazing effect on milk FA composition is due to higher 18:3n-3 intake on pasture. Although 18:3n-3 is extensively biohydrogenated in the rumen, more intermediates, e.g. c9-18:1, t11-18:1 and c9t11-8:2 in addition to the end-product 18:0, pass the rumen and are absorbed in the intestine.

Total FA and 18:3n-3 contents of grazed forage decrease with maturity (see Section 9.3.1), and pasture milk content of 18:3n-3 and c9t11-18:2 decreases accordingly. Typically, milk fat concentrations of 18:3n-3 and c9t11-18:2 from grazing cows are highest during spring and autumn and lower during summer when the grazed plants are in the generative stage (Bauchart *et al.*, 1984; Lock and Garnsworthy, 2003). Cows offered new, ungrazed pasture produce milk

with higher content of *c*9*t*11-18:2 than the cows following after, which can be related to difference in leaf blade proportion of the canopy and herbage 18:3*n*-3 content (Elgersma *et al.*, 2008).

A higher milk fat content of 18:3*n*-3 is often observed on hay diets than on silage-based diets (Shingfield *et al.*, 2005; Ferlay *et al.*, 2006; Chilliard *et al.*, 2007), and also when compared with grazing (Table 9.4). This is surprising as dietary content and intake of 18:3*n*-3 in these studies were much lower on hay due to oxidative losses of 18:3*n*-3 during hay wilting. The likely mechanism is that the biohydrogenation of 18:3*n*-3, for some reason, is stronger with fresh grass and silage diets than with hay diets, as observed *in vitro* for timothy hay compared with haylage and silage of timothy (Boufaied *et al.*, 2003b). No clear effect of silage additives has been found on milk FA composition, but Shingfield *et al.* (2005) found decreased levels of 18:3*n*-3 and increased levels of *c*9 *t*11-18:2 in milk when a formic acid-based product was used.

Botanical effects

Studies have shown that cows fed white or red clover silages yielded milk with higher contents of 18:2*n*-6 and 18:3*n*-3 (Table 9.5) and lower *n*-6/*n*-3 FA ratio than those fed on grass silages (Dewhurst *et al.*, 2003b; Al Mabruk *et al.*, 2004; Vanhatalo *et al.*, 2007; van Dorland *et al.*, 2008). In most comparisons, clover has been compared with ryegrass, but Vanhatalo *et al.* (2007) found similar results when red clover silage was compared with timothy-meadow fescue grass silage (Table 9.5). Clover had no consistent effect on milk FA synthesized *de novo* (not shown) or on *c*9-18:1, *c*9*t*11-18:2 and *t*11-18:1 (Table 9.5). Vanhatalo *et al.* (2007) found, however, that diets with red clover silage enhanced milk MUFA and PUFA and reduced concentrations of 10:0, 12:0, 14:0 and 16:0. The impact of red clover on milk 18:2*n*-6 and 18:3*n*-3 content could not be explained by the difference in FA intake as the ingestion was comparable across treatments (Dewhurst *et al.*, 2003b; Al Mabruk *et al.*, 2004; Vanhatalo *et al.*, 2007; van Dorland *et al.*, 2008). This was reflected in higher apparent transfer of 18:3*n*-3 and 18:2*n*-6 from the diet into milk for clover silages than for the corresponding grass silage diets. Similarly, Steinshamn and Thuen (2008) found higher apparent transfer efficiency of 18:2*n*-6 and 18:3*n*-3 on red clover than on white clover containing silage diets and a higher milk fat content of 18:2*n*-6, 18:3*n*-3 and PUFA and a lower *n*-6/*n*-3 ratio on red clover (Table 9.5). No differences between the two clover species have been observed in other studies (Table 9.5), probably due to a higher intake of 18:3*n*-3 on white clover than on red clover in these studies. It has been suggested that the mechanism for higher recovery of 18:3*n*-3 on red clover diets is through the action of the enzyme polyphenol oxidase (PPO). PPO inhibits lipolysis by producing electrophilic quinones, which to some extent protect the fatty acids from rumen biohydrogenation (Lee *et al.*, 2004). A higher recovery of 18:3*n*-3 on white clover diets than on grass diets is probably due to a higher ruminal passage rate, which reduces exposure of forage lipids to lipases and biohydrogenation (Dewhurst *et al.*, 2003a). However, the action of clover saponins has also been suggested, as reduced

Table 9.5 Effects of clover silages on concentrations of unsaturated fatty acids in milk (g/100 g)

Study (experiment no./ treatment)	Clover species and concentrate feeding level (kg DM/day)	18:2n-2		18:3n-3		c9t11-18:2		t11-18:1		c9-18:1	
		Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover
Dewhurst et al. (2003b)	T. repens (7)	1.42*	1.80	0.43*	0.91	–	–	–	–	24.7	20.0
Dewhurst et al. (2003b)	T. pratense (7)	1.42*	1.81	0.43*	0.81	–	–	–	–	24.7	24.0
Dewhurst et al. (2003b)	T. repens (7)	1.05*	1.54	0.40*	0.96	0.38	0.37	1.13	1.06	20.7*	17.9
Dewhurst et al. (2003b)	T. pratense (7)	1.05*	1.58	0.40*	1.28	0.38*	0.44	1.13*	1.25	20.7	20.2
Dewhurst et al. (2003b)	T. pratense (4)	0.90*	1.47[b]	0.48*	1.51[b]	0.39*	0.45	1.16*	1.31	19.0	20.0
Al Mabruk et al. (2004)	T. pratense (7)	1.24*	1.54	0.48*	0.92	0.48	0.43	1.31*	1.16	23.2	23.5
Van Dorland et al. (2008)	T. repens (4)	1.52	1.43	0.90[†]	1.14	0.92[†]	0.76	1.92	1.55	19.2*	16.7
Van Dorland et al. (2008)	T. pratense (5)	1.52	1.43	0.90[†]	1.04	0.92[†]	0.79	1.92	1.78	19.2*	17.1
Vanhatalo et al. (2007) (early cut)	T. pratense (9)	1.24*	1.80	0.41*	1.34	0.50	0.50	0.99*	0.85	16.1*	19.0
Vanhatalo et al. (2007) (late cut)	T. pratense (9)	1.32*	1.65[c]	0.37*	0.88[c]	0.53	0.56[c]	1.00*	0.97	17.3*	18.4
Steinshamn and Thuen (2008)	T. repens (0)		0.86		0.87		1.02		1.99		18.9
Steinshamn and Thuen (2008)	T. repens (8)		1.26		0.58		0.91		1.47		19.2
Steinshamn and Thuen (2008)	T. pratense (0)		0.89[a]		1.04[a]		1.02		2.01		19.9
Steinshamn and Thuen (2008)	T. pratense (8)		1.35[b]		0.69[b]		0.92[b]		1.60[b]		18.7

* Differences ($P < 0.05$) reported between grass and clover in the different studies (within study and line).
[†] Tendency of differences ($P < 0.1$) reported between grass and clover in the different studies (within study and line).
[a] Differences ($P < 0.05$) reported between clover species in the different studies (within study and column).
[b] Differences ($P < 0.05$) reported between concentrate level in the different studies (within study and column).
[c] Differences ($P < 0.005$) reported between growth stages (within study and column).

lipolysis has been observed for white clover silage versus ryegrass silage (Lourenço *et al.*, 2008).

Data from different sources have been used to compare botanically diverse versus grass-based diets (Chilliard *et al.*, 2007; Lourenço *et al.*, 2008). In both reviews, it was found that botanically diverse pastures or silages gave milk that was significantly richer in 18:3*n*-3 and poorer in some of the *de novo* synthesized FA. Chilliard *et al.* (2007) also found that botanically diverse alpine pastures yielded milk with higher content of *c*9-18:1, *t*11-18:1 and *c*9*t*11-18:2. The effect could not be explained by higher content and intake of *c*9-18:1 and 18:3*n*-3 in alpine pastures. It has been suggested that the alpine effect is due to botanical components that reduce rumen biohydrogenation (Leiber *et al.*, 2005), and there are reports on many different plant secondary metabolites that inhibit lipase activity (Dewhurst *et al.*, 2006; Lourenço *et al.*, 2008).

Chilliard *et al.* (2001) compared maize silage with grass silage by compiling data from different studies. They found that maize silage increased the proportion of 6-12 FA and 18:2*n*-6 at the expense of 16:0, 18:0 and 18:3*n*-3, which also is seen by direct comparisons in Table 9.4. These results are probably due to the fact that maize silage has a low content of C18:3*n*-3 and is rich in C18:2*n*-6 and *c*9-18:1.

Farming system effects
Milk from organic farms has a higher proportion of 18:3*n*-3 than conventionally produced milk (Jahreis *et al.*, 1997; Ellis *et al.*, 2006; Butler *et al.*, 2008; Collomb *et al.*, 2008). This elevating effect on 18:3*n*-3 may be due to both the increased use of legumes and higher forage:concentrate ratio in organic production. The effect of organic farming on the other FA is less consistent.

9.3.4   Effects of concentrate source on milk fat content and composition

Chemical composition and other nutritional characteristics vary among species of cereal grains, and they have different effects on milk fat content. It is well documented that oats give more milk but lower fat content than barley (Ekern *et al.*, 2003). Likewise, in a number of studies, grains that provide a high proportion of starch for digestion in the small intestine (like maize) are associated with increases in milk yield accompanied by a decrease in milk fat concentration (Reynolds, 2006). Feed fat content and composition is likely to be one factor influencing milk fat content, because *t*10*c*12-18:2 produced as an intermediate in rumen biohydrogenation is shown to have a specific negative effect on milk fat synthesis in the udder (Griinari and Bauman, 2006). The negative effect of increased glucose supply via absorption from the intestine (rumen-resistant starch) on fat synthesis in the udder is most probably explained by changes in insulin status that redirect energy to body fat retention (Reynolds, 2006).

The amount and composition of the fat fraction vary among grains (Table 9.2). However, the effect of these differences on milk FA composition is not well documented, with the exception of oats versus barley. Feeding of oat-based

concentrates results in lower milk fat content, increased content of 18:0, *c*9 18:1 and *c*9 *t*11 18:2 and decreased content of 12:0, 14:0 and 16:0 FA in milk fat as compared to barley based concentrates (Fearon *et al.*, 1996; Ekern *et al.*, 2003). Moreover, in the study of Ekern *et al.* (2003), replacing regular oats with high-fat oats strengthened the positive effect of oats on the nutritional quality of milk fat. Thus, oats with a high proportion of *c*9-18:1 and a favourable proportion between linoleic and linolenic FA (Table 9.2) are obviously an interesting source of fat to dairy cows.

The effect of increasing the concentrate intake on milk FA composition depends on the concentrate fat content and its FA profile. However, under most practical conditions, increasing the grain concentrate intake increases both the milk proportion of *de novo* synthesized FA and 18:2*n*-6 at the expense of 18:3*n*-3 and *c*9*t*11-18:2, as long as the concentrate intake is in the lower range (Bargo *et al.*, 2002; Dewhurst *et al.*, 2003b; Steinshamn and Thuen, 2008). This is probably a result of lower intake of 18:3*n*-3 due to the substitution effect of concentrate on roughage intake. High-concentrate diets, i.e. with more than 50–60% of concentrate in the daily ration, result in fat depression and milk with a low proportion of 14:0–18:0 and rich in 18:2*n*-6 and *trans*-FA (see review by Chilliard *et al.* 2007).

The use of either whole oilseeds or seed oils in the diet is an effective way of changing milk FA composition (Chilliard *et al.*, 2007). In most experiments, the inclusion of these oils or oilseeds in the diet of lactating dairy cows results in reduction of the levels of 4–16 milk fatty acids and an increase in the levels of one or more of the long-chain fatty acids 18:0, *c*9-18:1, 18:2*n*-2 or 18:3*n*-3 (Chilliard *et al.*, 2007). The individual fat sources have their own specific effect, essentially reflecting their FA composition. The high proportion of *c*9-18:1 in rapeseeds is reflected in the high content of this FA in the milk fat fraction (Fearon *et al.*, 2004). Characteristic for linseed oil is a high proportion of linolenic acid and only a small fraction of linoleic acid. Thus, feeding linseed oil increases the proportion of *n*-3 fatty acids and results in a more favourable ratio (higher) between *n*-3 and *n*-6 FA compared to other oilseeds (Chilliard *et al.*, 2007). Because linoleic acid is the main precursor for CLA production in the rumen, feeding fat sources with a high content of this FA also results in a high content of CLA in the milk fraction (Dhiman *et al.*, 2000; Bell *et al.*, 2006). However, care should be taken because unsaturated fatty acids are toxic to many of the species of rumen bacteria, particularly those that are involved in fibre digestion (Palmquist and Jenkins, 1980). The result is depressed fibre digestion and consequently fewer precursors for *de novo* fat synthesis, thus resulting in lower fat content. Additionally, *t*10*c*12-18:2 acid produced in the biohydrogenation process in the rumen may mediate a negative effect on *de novo* synthesis of fatty acids in the mammary gland (Griinari and Bauman, 2006).

Processing of oilseeds, e.g. extrusion, micronizing, roasting and formaldehyde treatment, has given variable results in affecting the milk FA composition when compared to raw seeds or oil (see review by Chilliard *et al.*, 2007).

9.4 Milk protein content and composition

Cow's milk normally contains in the order of 2.8–3.5% protein, but with considerable variations between breeds. The nitrogen fraction in milk is distributed among three major groups: caseins, whey proteins and non-protein nitrogen (NPN). These contribute to approximately 78.5, 16.5 and 5.0% of milk N, respectively, but there are distinctive breed differences (DePeters and Ferguson, 1992). Urea is the dominating contributor of the NPN fraction with about 50% (DePeters and Ferguson, 1992). Milk protein content as well as its composition vary with breed, and factors like stage of lactation and parity (DePeters and Ferguson, 1992). The effect of nutrition on the protein content and composition in bovine milk is discussed below.

9.4.1 Feeding factors influencing milk protein content and composition

The feeding regime has only a small impact on the relative distribution of the milk protein fractions and consequently on amino acid composition, and will therefore not be considered further in this review.

The synthesis of milk proteins within the udder requires a sufficient supply of essential amino acids, non-essential amino acids or precursors for synthesis of the latter as well as energy substrates. Because proteins have a genetically determined amino acid sequence, not only does the synthesis of milk protein require sufficient amino acids, they also have to be supplied in a predetermined ratio (Lapierre *et al.*, 2006). In most feeding situations, more than half, and often two-thirds to three-quarters, of the amino acids absorbed are derived from microbial protein (Dewhurst *et al.*, 2000). Moreover, rumen microbes have a variable, but generally good amino acid profile (Lapierre *et al.*, 2006). Consequently, the microbial protein synthesis in the rumen is of essential importance. However, in feeding situations with a rather high proportion of feed protein escaping degradation in the rumen (by-pass protein), the amino acid profile of that protein fraction is important (Lapierre *et al.*, 2006).

In general, milk protein content is relatively unresponsive to feeding factors. However, there is a relatively close positive correlation between energy supply and protein content in milk (Coulon and Remond, 1991). On most diets, the amount of microbial protein synthesized in the rumen increases with increasing intake (Dewhurst *et al.*, 2000). Accordingly, the apparently good correlation between energy supply and protein content in milk (Coulon and Remond, 1991) may be confounded by a protein effect as well. As milk yield also responds positively to these two factors, milk protein content is often elevated by increasing milk yield (Coulon and Remond, 1991; Huthanen and Rinne, 2006). Thus, a feeding strategy for obtaining high milk yields is probably the most efficient way of increasing milk protein content.

The positive correlation between energy supply and milk protein content also depends on energy source. It is well documented that milk protein content may be negatively influenced by high intake of dietary fat (DePeters and Ferguson, 1992). Thus, there may be a conflict between the aim of obtaining milk with

favourable FA composition and high protein content. It is not fully understood why feeding fat negatively influences milk protein content. Both ruminal and post-ruminal factors are probably involved (DePeters and Ferguson, 1992). Increasing the fat level in the diet often results in less carbohydrate and accordingly reduced microbial protein synthesis. Thus, without compensating that with more by-pass protein, the protein supply will decrease, which probably at least partly explains the specific negative effect of fat. Milk protein production may be limited by a shortage of glycogenic nutrients, which may cause amino acids to be used as precursors for the synthesis of glucose. From that perspective, more rumen-resistant starch should be beneficial. However, there is no clear evidence that the site of starch digestion has any effect on milk protein content (Nocek and Tamminga, 1991; Reynolds, 2006).

9.5   Content of vitamins

Apart from being essential for biological functions in the animal, vitamins are important for the nutritional value of milk and its shelf-life. Milk is a good dietary source of the water-soluble vitamins B_1 (thiamin, 0.044 mg per 100 g), B_2 (riboflavin, 0.183 mg/100 g) and B_{12} (cobalamin, 0.44 μg per 100 g) and the fat-soluble vitamins A (retinol, 28 μg per 100 g), D (40 IU) and E (α-tocopherol, 0.06 mg per 100 g) (content in whole milk with 3.25% milk fat adapted from the USDA Nutrient Database: http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl). Milk content of vitamin E and carotenoids, vitamin A precursors, are of particular interest as they are antioxidants and may affect the shelf-life of milk and milk products.

9.5.1 β-carotene and Vitamin A

In ruminants, as well as in other animals, vitamin A (retinol) is synthesized from carotenoids in the intestine and liver, and the principal source is forage carotenoids. The main form of carotenoids in milk is β-carotene (usually 85% of total carotenoid content) (Martin *et al.*, 2004; Havemose *et al.*, 2006; Noziere *et al.*, 2006). The β-carotene content in forage decreases with plant maturity and by wilting, and is lower in concentrates and maize silage than in forages. Milk concentration of β-carotene is directly related to the amount ingested by the cows (Martin *et al.*, 2004). Consequently, the β-carotene milk content is highest in milk produced on young pastures and higher on grass silage-based diets than on diets based on hay or maize silage (Havemose *et al.*, 2004; Martin *et al.*, 2004; Calderon *et al.*, 2007). Milk concentrations of vitamin A (retinol) are less affected by forage type and intake of its precursor β-carotene than milk content of β-carotene (Martin *et al.*, 2004; Noziere *et al.*, 2006). This is probably due to homeostatic regulation of production of vitamin A from β-carotene during absorption and vitamin A release by the liver (Noziere *et al.*, 2006).

9.5.2   Vitamin E

As for β-carotene, forage vitamin E (α-tocopherol) is destroyed by oxidation enhanced by ultraviolet radiation, as during wilting for silage and haymaking. The concentration is therefore lower in silage and hay than in fresh forage (Muller *et al.*, 2007), and vitamin E content is higher in milk from cows fed pasture or silage than in milk from cows fed either concentrate-rich diets, maize silage or hay (Havemose *et al.*, 2004; Martin *et al.*, 2004; Noziere *et al.*, 2006). The concentration in milk is closely related to the amount ingested by the cows, as long as the vitamin E source is natural (Martin *et al.*, 2004; Calderon *et al.*, 2007). α-Tocopherol exists in eight different stereo-isomers, of which RRR-α-tocopherol has the highest biological activity and is the only isomer found in plants.

9.5.3   Effect of β-carotene and vitamin E on milk shelf-life

Milk produced from animals grazing fresh forage or on a grass silage-based ration has high contents of vitamin E, β-carotene and other compounds that are believed to act as antioxidants and positively influence the shelf-life of these products (Havemose *et al.*, 2004; Martin *et al.*, 2004; Agabriel *et al.*, 2007; Butler *et al.*, 2008). Thus, supplementation with vitamin E or β-carotene to grazing dairy cows should not be necessary. However, despite higher or similar milk vitamin E content, poorer milk lipid oxidative stability has been found on grass–clover silage rations than on maize or hay rations (Havemose *et al.*, 2004, 2006). The likely reason is that grass–clover silage-based diets also increase the milk fat content of C18:3n-3 that is more exposed to oxidation than milk with a lower degree of FA unsaturation. High levels of tocopherols and β-carotene have, however, proved to delay milk protein oxidation (Havemose *et al.*, 2004).

Supplementation with high amounts of vitamin E subsequently increases the content of α-tocopherol in milk (St-Laurent *et al.*, 1990; Charmley and Nicholson, 1994; Al Mabruk *et al.*, 2004), and some authors recommend very high dietary doses to improve quality (McDowell *et al.*, 1996). However, the effect of supplementation with vitamin E on the oxidative stability of milk lipids is not unequivocal. Supplementation has shown positive effect (Al Mabruk *et al.*, 2004), no effect (Charmley and Nicholson, 1994; Havemose *et al.*, 2004), or even negative effect (Slots *et al.*, 2007). A poor response can partly be explained by a very low transfer rate of synthetic stereo-isomers. Irrespective of supplementary source and stereo-isomer, nearly all (>84%) α-tocopherol found in milk is the natural stereo-isomer RRR-α-tocopherol (Meglia *et al.*, 2006; Slots *et al.*, 2007). Another reason for the poor effect of vitamin E supplementation is that, in milk with high levels of α-tocopherol and unsaturated fat, α-tocopherol might become pro-oxidative (Slots *et al.*, 2007). Although there is a direct relation between intake and milk content of α-tocopherol and β-carotene, the secretion of these compounds is not limited by the amount arriving to the mammary gland by plasma but by the transfer from plasma to milk (Jensen *et al.*, 1999; Calderon *et al.*, 2007). The transfer from plasma to milk follows Michaelis–Menten

kinetics for active transport across membranes, and the daily secretion is therefore limited in quantity (Jensen *et al.*, 1999). This means that increasing milk and milk fat yields result in a dilution of these vitamins in milk. However, it seems that the variation in the fatty acid profile is more important for the oxidative stability of milk than its content of α-tocopherol and β-carotene, but this warrants further research.

9.6   Content of minerals

Bovine milk contains a wide range of minerals. Of these, selenium and iodine are of special interest because they are of great importance in human nutrition (Haug *et al.*, 2007), and are significantly altered by the feeding regime.

9.6.1   Selenium
The milk content of selenium varies worldwide. In South Dakota the selenium concentration in milk is reported to be between 160 and 1300 μg/l, whereas the concentrations in milk from low-selenium regions may be from 5 to 30 μg/l (Haug *et al.*, 2007), as in Scandinavia and northern Europe. Besides the selenium concentration in the feed, the source of selenium is of great importance. Milk selenium concentrations are more than twice as high when selenium yeast is fed compared with selenite or selenate (Weiss, 2003; Givens *et al.*, 2004).

9.6.2   Iodine
Up to about 25% of the iodine intake is normally excreted in milk (Crout and Voigt, 1996). Therefore, the milk content of iodine also varies depending on the iodine content and availability in the feeds used. A study on milk and milk products in Norway (Dahl *et al.*, 2003) showed that milk from the summer season had a significantly lower iodine concentration (88 μg/l) than milk from the winter season (232 μg/l). This is explained by the use of more supplementary feeds enriched with iodine during the winter season. As the feed industry adds iodine to cattle feed, dairy products supply much of the dietary intake of iodine. In Norway, dairy iodine represents the largest single iodine source, and in USA the second-largest source of dietary iodine (Haug *et al.*, 2007).

9.7   Conclusions and practical implications

Feeding strategies for improving the nutritional quality of milk are summarized in Table 9.6. There are strong seasonal variations in the nutritional quality of milk. The fat fraction in milk from grazing dairy cows has a more favourable FA composition and contains significantly more vitamin A and E than milk

Table 9.6 Summary of the effects of different feeding strategies on the nutritional quality of milk

Milk component[a]	Possibility for modification[b]	Feeding strategies/feeding systems[a]
More favourable FA composition:		
↓ Saturated FA	Minor to	↑ Pasture vs. silages
↑ Unsaturated FA	considerable	↑ Grass silage vs. maize silage
↑ *n*-3/*n*-6 FA ratio		↑ Oats vs. barley
↑ *c9t*11-18:2 (CLA)		↑ Botanical diverse forage vs. pure species of grasses
Specific effects:		
↑ *n*-3/*n*-6 FA ratio	Minor to moderate	↑ Hay vs. silage
		↑ Red clover silage vs. grass silage
		↑ White clover silage vs. grass silage
		↑ Botanical diverse forage vs. pure species of grasses
		↑ Oils or oilseeds with high proportion of *n*-3 FA
↑ *c9t*11-18:2 (CLA)	Considerable	↑ Oils or oilseeds with high proportion of *n*-6 FA
		↑ Grazing plants in young stage of maturity
↑ Protein	Minor	↑ Intake of energy
		↑ Intake of dietary by-pass protein
		↑ Moderate intake of fat
		↑ Favourable conditions for microbial protein synthesis
↑ Vitamin A	Considerable	↑ Roughage; early stage of maturity at harvesting
		↑ Pasture vs. silages
		↑ Grass silage vs. maize silage
		↑ Concentrates with naturally high content of vitamin A
		↑ Vitamin A supplementation
↑ Vitamin E	Considerable	↑ Roughage; early stage of maturity at harvesting
		↑ Pasture vs. silages
		↑ Grass silage vs. maize silage
		↑ Concentrate with naturally high content of vitamin E
		↑ Vitamin E supplementation
↑ Selenium	Considerable	↑ Feeds with naturally high content of selenium
		↑ Selenium supplementation
↑ Iodine	Considerable	↑ Feeds with naturally high content of iodine
		↑ Iodine supplementation

[a] ↓ Decrease; ↑ increase.
[b] Minor, ~25% change; moderate, ~25–100% change; considerable, ~>100% change.

produced on traditional indoor feeding composed of concentrate and conserved roughages. On indoor feeding, silage of grasses gives rise to a more favourable FA composition and a higher content of vitamin A and E than maize silage. Use of hay or silage of clover instead of grass silage will further increase milk content of 18:3n-3. Intake of botanical diverse forages elevates the milk fat proportion of c9t11-18:2 (CLA) in addition to 18:3n-3. The ingredients of concentrate supplements also influence milk FA composition. Substitution of barley with oats influences FA composition positively. Likewise, proper use of oils or oilseeds in the diet will give rise to a more favourable milk FA composition. Feeding strategies that increase intake of energy and the delivery of amino acids to the small intestine are the most efficient way to increase milk protein content. The most efficient way to increase milk content of especially selenium and iodine, but also vitamins A and E, is to include them in supplementary feeds.

9.8   References

AGABRIEL C, CORNU A, JOURNAL C, SIBRA C, GROLIER P, MARTIN B (2007) Tanker milk variability according to farm feeding practices: Vitamins A and E, carotenoids, color, and terpenoids. *J Dairy Sci*, 90, 4884–4896.

AL MABRUK RM, BECK NFG, DEWHURST RJ (2004) Effects of silage species and supplemental vitamin E on the oxidative stability of milk. *J Dairy Sci*, 87, 406–412.

ARVIDSSON K, GUSTAVSSON A-M, MARTINSSON K (2009) Effects of conservation method on fatty acid composition of silage. *Anim Feed Sci Technol*, 148, 241–252.

BARGO F, MULLER LD, DELAHOY JE, CASSIDY TW (2002) Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. *J Dairy Sci*, 85, 1777–1792.

BARGO F, DELAHOY JE, SCHROEDER GF, BAUMGARD LH, MULLER LD (2006) Supplementing total mixed rations with pasture increase the content of conjugated linoleic acid in milk. *Anim Feed Sci Technol*, 131, 226–240.

BAUCHART D, VERITE R, REMOND B (1984) Long-chain fatty acid in lactating cows fed fresh grass from spring to autumn. *Can J Anim Sci*, 64 (Suppl.), 330–331.

BELL JA, GRIINARI JM, KENNELLY JJ (2006) Effect of safflower oil, flaxseed oil, monensin, and vitamin E on concentration of conjugated linoleic acid in bovine milk fat. *J Dairy Sci*, 89, 733–748.

BOUFAIED H, CHOUINARD PY, TREMBLAY GF, PETIT HV, MICHAUD R, BELANGER G (2003a) Fatty acids in forages. I. Factors affecting concentrations. *Can J Anim Sci*, 83, 501–511.

BOUFAIED H, CHOUINARD PY, TREMBLAY GF, PETIT HV, MICHAUD R, BELANGER G (2003b) Fatty acids in forages. II. In vitro ruminal biohydrogenation of linolenic and linoleic acids from timothy. *Can J Anim Sci*, 83, 513–522.

BUTLER G, NIELSEN JH, SLOTS T, EYRE MD, SANDERSON R, LEIFERT C (2008) Fatty acid and fat-soluble antioxidant concentrations in milk from high- and low-input conventional and organic systems: seasonal variation. *J Sci Food Agric*, 88, 1431–1441.

CALDERON F, CHAUVEAU-DURIOT B, PRADEL P, MARTIN B, GRAULET B, DOREAU M, NOZIERE P

(2007) Variations in carotenoids, vitamins A and E, and color in cow's plasma and milk following a shift from hay diet to diets containing increasing levels of carotenoids and vitamin E. *J Dairy Sci*, 90, 5651–5664.

CHARMLEY E, NICHOLSON JWG (1994) Influence of dietary-fat source on oxidative stability and fatty-acid composition of milk from cows receiving a low or high level of dietary Vitamin-E. *Can J Anim Sci*, 74, 657–664.

CHILLIARD Y, FERLAY A, MANSBRIDGE RM, DOREAU M (2000) Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. *Ann Zootech*, 181–205.

CHILLIARD Y, FERLAY A, DOREAU M (2001) Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livest Prod Sci*, 70, 31–48.

CHILLIARD Y, GLASSER F, FERLAY A, BERNARD L, ROUEL J, DOREAU M (2007) Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *Eur J Lipid Sci Technol*, 109, 828–855.

COLLOMB M, BISIG W, BÜTIKOFER U, SIEBER R, BREGY M, ETTER L (2008) Fatty acid composition of mountain milk from Switzerland: Comparison of organic and integrated farming systems. *Int Dairy J*, 18, 976–982.

COULON JB, REMOND B (1991) Variations in milk output and milk protein-content in response to the level of energy supply to the dairy cow – a review. *Livest Prod Sci*, 29, 31–47.

CROUT NM, VOIGT G (1996) Modeling the dynamics of radioiodine in dairy cows. *J Dairy Sci*, 79, 254–259.

DAHL L, OPSAHL JA, MELTZER HM, JULSHAMN K (2003) Iodine concentration in Norwegian milk and dairy products. *Br J Nutr*, 90, 679–685.

DEPETERS EJ, FERGUSON JD (1992) Nonprotein nitrogen and protein distribution in the milk of cows. *J Dairy Sci*, 75, 3192–3209.

DEWHURST RJ, KING PJ (1998) Effects of extended wilting, shading and chemical additives on the fatty acids in laboratory grass silages. *Grass Forage Sci*, 219–224.

DEWHURST RJ, DAVIES DR, MERRY RJ (2000) Microbial protein supply from the rumen. *Anim Feed Sci Technol*, 85, 1–21.

DEWHURST RJ, SCOLLAN ND, YOUELL SJ, TWEED K, HUMPHREYS MO (2001) Influence of species, cutting date and cutting interval on the fatty acid composition of grasses. *Grass Forage Sci*, 56, 68–74.

DEWHURST RJ, EVANS RT, SCOLLAN ND, MOORBY JM, MERRY RJ, WILKINS RJ (2003a) Comparison of grass and legume silages for milk production. 2. In vivo and in sacco evaluations of rumen function. *J Dairy Sci*, 86, 2612–2621.

DEWHURST RJ, FISHER WJ, TWEED JKS, WILKINS RJ (2003b) Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. *J Dairy Sci*, 86, 2598–2611.

DEWHURST RJ, SHINGFIELD KJ, LEE MRF, SCOLLAN N (2006) Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Anim Feed Sci Technol*, 131, 168–206.

DHIMAN TR, ANAND GR, SATTER LD, PARIZA MW (1999) Conjugated linoleic acid content of milk from cows fed different diets. *J Dairy Sci*, 82, 2146–2156.

DHIMAN TR, SATTER LD, PARIZA MW, GALLI MP, ALBRIGHT K, TOLOSA MX (2000) Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. *J Dairy Sci*, 83, 1016–1027.

DOREAU M, FERLAY A (1994) Digestion and utilization of fatty-acids by ruminants. *Anim Feed Sci Technol*, 45, 379–396.

EKERN A, HAVREVOLL O, HAUG A, BERG J, LINDSTAD P, SKEIE S (2003) Oat and barley based concentrate supplements for dairy cows. *Acta Agric Scand Sect A – Anim Sci*, 53, 65–73.

ELGERSMA A, ELLEN G, VAN DER HORST H, MUUSE BG, BOER H, TAMMINGA S (2003) Comparison of the fatty acid composition of fresh and ensiled perennial ryegrass (*Lolium perenne* L.), affected by cultivar and regrowth interval. *Anim Feed Sci Technol*, 108, 191–205.

ELGERSMA A, ELLEN G, VAN DER HORST H, BOER H, DEKKER PR, TAMMINGA S (2004) Quick changes in milk fat composition from cows after transition from fresh grass to a silage diet. *Anim Feed Sci Technol*, 117, 13–27.

ELGERSMA A, MAUDET P, WITKOWSKA IM, WEVER AC (2005) Effects of nitrogen fertilisation and regrowth period on fatty acid concentrations in perennial ryegrass (*Lolium perenne* L.). *Ann Appl Biol*, 147, 145–152.

ELGERSMA A, VAN DER HOEVEN E, WITKOWSKA I, SMIT HJ (2008) Effects of the grazed horizon in perennial ryegrass swards on the cojugated linoleic acid concentration in milk of dairy cows. *Grassl Sci Eur*, 13, 388–389.

ELLIS KA, INNOCENT G, GROVE-WHITE D, CRIPPS P, MCLEAN WG, HOWARD CV, MIHM M (2006) Comparing the fatty acid composition of organic and conventional milk. *J Dairy Sci*, 89, 1938–1950.

FEARON AM, MAYNE CS, MARSDEN S (1996) The effect of inclusion of naked oats in the concentrate offered to dairy cows on milk production, milk fat composition and properties. *J Sci Food Agric*, 72, 273–282.

FEARON AM, SINCLAIR MAYNE C, BEATTIE JAM, BRUCE DW (2004) Effect of level of oil inclusion in the diet of dairy cows at pasture on animal performance and milk composition and properties. *J Sci Food Agric*, 84, 497–504.

FERLAY A, MARTIN B, PRADEL P, COULON JB, CHILLIARD Y (2006) Influence of grass-based diets on milk fatty acid composition and milk lipolytic system in Tarentaise and Montbeliarde cow breeds. *J Dairy Sci*, 89, 4026–4041.

GIVENS D I, ALLISON R, COTTRILL B, BLAKE JS (2004) Enhancing the selenium content of bovine milk through alteration of the form and concentration of selenium in the diet of the dairy cow. *J Sci Food Agric*, 84, 811–817.

GRIINARI J M, BAUMAN DE (2006) 'Regulation of milk fat production', in *Ruminant Physiology: Digestion, Metabolism, and Impact of Gene Expression, Immunology and Stress*. Sejrsen K, Hvelplund T, Nielson MO (eds), Wageningen Academic Publishers, Wageningen, The Netherlands, 383–411.

GRIINARI JM, CORL BA, LACY SH, CHOUINARD PY, NURMELA KVV, BAUMAN DE (2000) Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Delta(9)-desaturase. *J Nutr*, 130, 2285–2291.

HARFOOT CV, HAZLEWOOD GP (1997) 'Lipid metabolism in the rumen', in *The Rumen Microbial Ecosystem*, Blackie, London, 382–426.

HAUG A, HØSTMARK AT, HARSTAD OM (2007) Bovine milk in human nutrition – a review. *Lipids Health Dis*, 6, 25–41.

HAVEMOSE MS, WEISBJERG MR, WENDER WLP, BREDIE LP, NIELSEN JH (2004) Influence of feeding different types of roughage on the oxidative stability of milk. *Int Dairy J*, 14, 563–570.

HAVEMOSE MS, WEISBJERG MR, BREDIE WLP, POULSEN HD, NIELSEN JH (2006) Oxidative stability of milk influenced by fatty acids, antioxidants, and copper derived from

feed. *J Dairy Sci*, 89, 1970–1980.

HAWKE JC (1973) 'Lipids', in *Chemistry and Biochemistry of Herbage*, Butler UW and Bailey RW (eds), Academic Press, London, 213–263.

HUTHANEN P, RINNE M (2006) Effects of increasing the milk yield of dairy cows on milk composition. *J Anim Feed Sci*, 16 (Suppl 1), 42–58.

JAHREIS G, FRITSCHE J, STEINHART H (1997) Conjugated linoleic acid in milk fat: High variation depending on production system. *Nutr Res*, 17, 1479–1484.

JENSEN SK, JOHANNSEN AKB, HERMANSEN JE (1999) Quantitative secretion and maximal secretion capacity of retinol, beta-carotene and alpha-tocopherol into cows' milk. *J Dairy Res*, 66, 511–522.

KAYLEGIAN KE, LINDSAY RC (1995) 'Milk fat usage and modification', in *Handbook of Milkfat Fractionation Technology and Application*, Kaylegian KE, Lindsay RC (eds), AOCS Press, Champaign, IL, 1–18.

KURTZ FE (1978) 'The lipids of milk: composition and properties', in *Fundamentals of Dairy Chemistry*, 2nd edn, Webb BH, Johnson AH, Alford JA (eds), Avi Publishing, Westport, CT, 125–219.

LAPIERRE H, PACHECO D, BERTHIAUME R, OUELLET DR, SCHWAB CG, DUBREUIL P, HOLTROP G, LOBLEY GE (2006). What is the true supply of amino acids? *J Dairy Sci*, 89 (E Suppl), E1–E14.

LEE MRF, WINTERS AL, SCOLLAN ND, DEWHURST RJ, THEODOROU MK, MINCHIN FR (2004) Plant-mediated lipolysis and proteolysis in red clover with different polyphenol oxidase activities. *J Sci Food Agric*, 84, 1639–1645.

LEIBER F, KREUZER M, NIGG D, WETTSTEIN HR, SCHEEDER MRL (2005) A study on the causes for the elevated *n*-3 fatty acids in cows' milk of alpine origin. *Lipids*, 40, 191–202.

LOCK AL, GARNSWORTHY PC (2003) Seasonal variation in milk conjugated linoleic acid and D^9-desaturase activity in dairy cows. *Livest Prod Sci*, 79, 47–59.

LOURENÇO M, VAN RANST G, VLAEMINCK B, DE SMET S, FIEVEZ V (2008) Influence of different dietary forages on the fatty acid composition of rumen digesta as well as ruminant meat and milk. *Anim Feed Sci Technol*, 145, 418–437.

MARTIN B, FEDELE E, FERLAY A, GROLIER P, ROCK E, GRUFFAT D, CHILLARD Y (2004) Effects of grass-based diets on the content of micronutrients and fatty acids in bovine and caprine dairy products. *Grassl Sci Eur*, 9, 876–886.

MCDONALD P, EDWARDS RA, GREENHALGH JFD, MORGAN CA (2002) *Animal Nutrition*, 6th edn, Prentice Hall, Harlow, UK.

MCDOWELL LR, WILLIAMS SN, HIDIROGLOU N, HILL GM, OCHOA L, WILKINSON NS (1996) Vitamin E supplementation for the ruminant. *Anim Feed Sci Technol*, 60, 273–296.

MEGLIA GE, JENSEN SK, LAURIDSEN C, WALLER KP (2006) Alpha-tocopherol concentration and stereoisomer composition in plasma and milk from dairy cows fed natural or synthetic vitamin E around calving. *J Dairy Res*, 73, 227–234.

MULLER CE, MOLLER J, JENSEN SK, UDEN P (2007) Tocopherol and carotenoid levels in baled silage and haylage in relation to horse requirements. *Anim Feed Sci Technol*, 137, 182–197.

NOCEK J E, TAMMINGA S (1991) Site of digestion of starch in the gastrointestinal tract of dairy cows and its effects on milk yield and composition. *J Dairy Sci*, 74, 3598–3629.

NORWEGIAN FEED TABLE (2008) http://www.umb.no/iha/fortabell/index.php, accessed 4 October 2008.

NOZIERE P, GROLIER P, DURAND D, FERLAY A, PRADEL P, MARTIN B (2006) Variations in carotenoids, fat-soluble micronutrients, and color in cows' plasma and milk

following changes in forage and feeding level. *J Dairy Sci*, 89, 2634–2648.

PALMQUIST DL, JENKINS TC (1980) Fat in lactation rations: Review. *J Dairy Sci*, 63, 1–14.

PALMQUIST DL, BEAULIEU AD, BARBANO DM (1993) Feed and animal factors influencing milk-fat composition. *J Dairy Sci*, 76, 1753–1771.

REYNOLDS CK (2006) Production and metabolic effects of site of starch digestion in lactating dairy cattle. *Anim Feed Sci Technol*, 130, 78–94.

SHINGFIELD KJ, SALO-VAANANEN P, PAHKALA E, TOIVONEN V, JAAKKOLA S, PIIRONEN V, HUHTANEN P (2005) Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of cows' milk. *J Dairy Res*, 72, 349–361.

SJAASTAD ØV, HOVE K, SAND O (2003), *Physiology of Domestic Animals*, Scandinavian Veterinary Press, Oslo.

SLOTS T, SKIBSTED LH, NIELSEN JH (2007) The difference in transfer of all-rac-alpha-tocopherol stereo-isomers to milk from cows and the effect on its oxidative stability. *Int Dairy J*, 17, 737–745.

ST-LAURENT AM, HIDIROGLOU M, SNODDON M, NICHOLSON JWG (1990) Effect of alpha-tocopherol supplementation to dairy-cows on milk and plasma alpha-tocopherol concentrations and on spontaneous oxidized flavor in milk. *Can J Anim Sci*, 70, 561–570.

STEINSHAMN H, THUEN E (2008) White or red clover-grass silage in organic dairy milk production: Grassland productivity and milk production responses with different levels of concentrate. *Livest Sci*, 119, 202–215.

VAN DORLAND HA, KREUZER M, LEUENBERGER H, WETTSTEIN H-R (2008) Comparative potential of white and red clover to modify the milk fatty acid profile of cows fed ryegrass-based diets from zero-grazing and silage systems. *J Sci Food Agric*, 88, 77–85.

VAN RANST G, FIEVEZ V, DE RIEK J, VAN BOCKSTAELE E (2009) Influence of ensiling forages at different dry matters and silage additives on lipid metabolism and fatty acid composition. *Anim Feed Sci Technol*, 150, 62–74.

VANHATALO A, KUOPPALA K, TOIVONEN V, SHINGFIELD KJ (2007) Effects of forage species and stage of maturity on bovine milk fatty acid composition. *Eur J Lipid Sci Technol*, 109, 856–867.

WEISS WP (2003) 'Selenium nutrition of dairy cows: Comparing responses to organic and inorganic selenium forms', in *Proc 19th Alltech Annual Symp Nutr, Biotechnol Feed Food Ind*, Lyons PT, Jacques KA (eds), Nottingham University Press, Nottingham, UK, 333–343.

WHITE PJ (2000) 'Fatty acids in oilseeds (vegetable oils)', in *Fatty Acids in Foods and their Health Implications*, Chow CK (ed.), Marcel Dekker, New York, 209–238.

10

Mastitis and raw milk quality, safety and yield

J. Hamann, University of Veterinary Medicine Hannover Foundation, Germany

Abstract: This chapter discusses the interaction between bovine mastitis and potential consequences with regard to milk quality, milk safety and the economic implications for the dairy farmer. Milk is a biological foodstuff with a high image concerning its quality and safety. Therefore the interaction between mastitis and hygienic and compositional milk quality is reviewed along with the most relevant aspects of mastitis control to show that milk producers are able to maintain a high milk quality not only now but also under future production conditions.

Key words: mastitis, milk quality, milk safety, economic losses, mastitis control.

10.1 Introduction

Mastitis, a multifactorial infectious disease of the mammary gland of dairy cows, occurs in the majority of cows at least once a year. The most important effects of mastitis consist of changes to the quality and safety of raw milk and milk yield reduction. Therefore, this chapter describes these interactions based on current knowledge. Section 10.2 details the different influences of mastitis on raw milk quality, safety and milk yield. To determine the respective level of mastitis influence on these three parameters, all data were compared with those of healthy udder quarters or mammary glands. In addition, the health risk to consumers of raw milk is compared with that of pasteurized milk. Some data will show the interaction between milk cell count level and milk yield per cow.

Information in Section 10.3 relates to the main causes of bovine mastitis with special regard to types of pathogens, movement of infection, invasion and infection, inflammation and susceptibility. It will be seen that generally the increase in milk yield over the last 10 years is not responsible for the actual mastitis situation. The elements of mastitis prevention and control are briefly discussed in Section 10.4. It can be concluded that the application of the somatic milk cell count level seems to be an excellent indicator for the introduction of mastitis control strategies. Sections 10.5 and 10.6 deal with future trends in dairying and sources of further information and advice.

10.2   Effects of mastitis on raw milk quality, safety and yield

The main effects of bovine mastitis result in changes to raw milk quality, safety and milk yield. The ecological, therapeutic and economic consequences of mastitis are not considered in this chapter.

10.2.1   Raw milk quality

Generally speaking, the term quality evokes as a relative concept in the majority of consumers a positive feeling concerning the product's properties. Related to the ISO 9000 (1994) definitions, it can be stated that quality can be determined by comparison of a set of inherent parameters with a set of requirements. High quality is achieved if the inherent characteristics meet all requirements. Therefore, depending on legislative regulations, milk production conditions and consumer demands, the type of parameters and their threshold values for quality scores will vary. The quality of a product is always relative, because it depends on the agreement with a special set of requirements. Consequently, no absolute value of quality is possible. In this chapter, milk quality is considered only in regard to hygienic, compositional and consumer-relevant aspects.

   Raw milk is not very well defined. One definition is given by Council Directive 92/46/EEC of 16 June 1992 in which under Article 2 it is stated: 'Milk produced by secretion of the mammary glands of one or more cows, ewes, goats or buffaloes, which has not been heated beyond 40ºC or undergone any treatment that has an equivalent effect'. Thus, all milk extracted from the mammary glands that is not heated above 40ºC can be characterized as raw milk (Wiesner and Ribbeck, 2000).

Hygienic quality of raw milk
The main criteria of hygienic quality of raw milk are the total bacterial count (TBC), the absence of pathogenic bacteria, and chemical residues and contaminant levels below the maximum residue limits (MRL) (Hamann, 2002a). Milk of good hygienic quality shows the following criteria (Ruegg and Tabone, 2000):

- low or very low numbers of saprophytic microorganisms (spoilage agents)
- absence or low numbers of pathogenic microbes
- absence of chemotherapeutic residues
- reduction or minimization of chemical contaminants.

This section will deal mainly with the two key parameters regarding mastitis: pathogenic microbes and chemotherapeutic residues.

Healthy udder quarters contain sterile milk. This statement was confirmed by results of experimental studies with aseptic milking via implanted cannulae in the bovine mammary gland, which indicated that sterile milk could be withdrawn up to 272 lactation days (Tolle and Heeschen, 1975). Yet, it is clear that also under conditions of healthy quarters a certain unavoidable contamination with bacteria will happen if the milk stream is passing through the external part of the teat canal orifice. This region is always contaminated with different types of microorganisms. Therefore, microbial contamination of 100–1000 microorganisms/ml of milk seems unavoidable. Any further increase of microbial counts in milk is due to mastitis (interior of the udder), external contamination of teats and udder, and/or contaminated surfaces of milking equipment or functionality of storage equipment, e.g. for cooling.

Milk of clinical mastitis cases, initiated by pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli* or others, may show, on average, bacterial counts of less than 100,000/ml milk, but occasionally the count may exceed several million bacteria/ml. Under such conditions only a single clinical mastitis case is needed to increase significantly the bacterial count of the herd bulk milk (Bramley, 1992).

The most important factor contributing to the bacterial count is the type of cleaning and disinfection of all surfaces of the milking plant that are in contact with milk during milking. Particularly, in terms of mastitis problem herds, such a procedure is of the utmost importance since it effectively removes milk residues and kills bacteria on the surfaces. Modern milking systems are fitted with excellent cleaning and disinfection systems, but such systems also need to undergo professional testing at predetermined intervals.

Worldwide, milk hygiene criteria such as the total bacterial count (TBC) are taken into consideration in special legal rules and/or corresponding payment schemes. The dairy farmer thus will have an economic interest in maintaining hygienic quality in such a range that will assure the premium-class price level. Cleaning and disinfection measures, proper machine milking procedures and appropriate cooling of the milk make it possible that under modern dairying conditions most milk produced falls into premium-class thresholds. As an example, the geometric mean bacterial contamination of all herd bulk milk samples in so-called recorded herds was 18,000 bacteria/ml during 2006 and 2007 in Germany (ADR, 2008).

A further aspect of hygienic quality of raw milk related to mastitis relates to the potential occurrence of chemotherapeutic residues in milk, especially after antibiotic treatment of clinical mastitis cases or after antibiotic infusion at the

end of the current lactation (dry-off therapy). The intramammary infusion of antibiotics as mastitis therapy is the most important source of antibiotic milk contamination (Mitchell *et al.*, 1998). The potential importance of antibiotic residues in milk consists of two main aspects, public health concerns and manufacturing problems. Concerning the public health aspects the following criteria have to be considered: development and/or transmission of antibiotic-resistant bacteria (Allison, 1985; Mitchell *et al.*, 1998), allergic reactions of milk consumers (Dewdney and Edwards, 1984), and effects on the gut flora (Allison, 1985; Langford *et al.*, 2003). It has to be stressed that also in pasteurized milk a certain level of antibiotic residues may exist (Moats, 1999). The second impairing effect of antibiotic residues relates to manufacturing problems, as antibiotics can inhibit cheese and yoghurt starter cultures (Cogan, 1972).

Due to legislative regulations, payment schemes and special agreements between milk producers and dairy factories, the frequency of antibiotic residue-positive milk samples has been reduced significantly worldwide, especially over the last 10 years. The overall occurrence of positive samples may well have been reduced by more than 50% (from 0.1% to less than 0.05%) (Dalton, 2006; BVL, 2007). To enable sustainable and economic dairying, however, an even greater reduction seems necessary. Overall, the main reason for milk being contaminated with antibiotics is human failure concerning communication of information about treatments of cows, calving of cows, withdrawal periods, etc. (Vassallo, 2005).

Compositional quality of raw milk
A large amount of data has been published concerning milk components that are used as criteria for milk payment schemes (e.g. fat, protein, somatic cells). In contrast, other milk components such as lactose, lactate, enzymes, fatty acids, etc., are not intensively characterized in the literature.

One parameter, the somatic cell count, is included in both the quality definition of milk and the health definition of udder quarters (IDF, 1967; DVG, 1994). After a long discussion within and between national and international commissions (FDA, IDF, DVG, etc.), it can be stated that the normal cell count of a healthy mammary quarter is approximately 100,000 cells per ml of milk (Hamann, 2002b; Smith, 2002).

The somatic cell count level can also be applied as an indicator for udder health and related changes in milk composition if the udder health is disturbed. Thus, two aspects should be regarded: cell count level and changes in milk composition, and cell count level and the occurrence of antibiotic residues in milk.

So long as the cell count amounts to less than 100,000 cells per ml milk secreted in a healthy quarter, the milk composition can be characterized as physiological (lies within the normal range). This statement has been confirmed by various researchers (Tolle *et al.*, 1971; Reichmuth, 1975; Barbano, 1999). One study reported the values of 19 milk constituents out of quarter composite milk samples from healthy udder quarters in German Holstein cows (cell count <

100,000/ml; no pathogens detectable) (Hamann *et al.*, 2002). The measurements were performed throughout 1.5 lactations per cow, so that at least 18 samples per quarter and lactation could be collected. Table 10.1 summarizes results for two different lactation stages (days in milk, or DIM) at DIM 10 and at DIM 210 of around 5000 quarter composite milk samples.

With the exception of the parameters urea, ß-hydroxybutyrate, lactose, fat, protein and potassium, all other parameters indicated a lactation stage-related significant difference ($P \leq 0.05$) between DIM 10 and DIM 210. This implies that for the majority of the measured milk components lactationally corrected reference values are required for proper evaluation of such results.

So long as the blood–udder barrier shows a physiological integrity, the number of somatic cells per ml of milk is below 100,000. Doggweiler and Hess (1983) reported a somatic cell count mean of 22,000/ml milk for approximately 3000 healthy quarters. Using twice the standard deviation as a normal safety limit will result in a maximal value for 'normal secretion' of 100,000 cells/ml. As early as 1971, Tolle *et al.* studied the relationship between somatic cell count and milk composition (Na, K, Cl, milk-N and lactose). Their results show that

Table 10.1   Physiological reference values of different milk constituents at DIM 10 and DIM 210

Parameter	Physiological level at DIM 10	Physiological level at DIM 210
Integrity of the blood–milk barrier		
pH	6.65	6.76
Electrical conductivity	4.88 mS/cm	5.11 mS/cm
Potassium	42.75 mmol/l	42.50 mmol/l
Sodium	14.79 mmol/l	13.80 mmol/l
Chloride	25.31 mmol/l	30.06 mmol/l
Lactate	32.60 μmol/l	45.50 μmol/l
Pyruvate	6.17 μmol/l	6.61 μmol/l
Integrity of the secretory epithelium		
Glucose	326.96 μmol/l	443.80 μmol/l
Galactose	199.53 μmol/l	724.44 μmol/l
Phosphoenolpyruvate	84.37 μmol/l	6.01 μmol/l
Citrate	9.51 μmol/l	8.41 μmol/l
Lactose	4.84%	4.71%
Fat	4.97%	4.81%
Protein	3.39%	3.33%
Immune status of the gland		
Somatic cells	13.8×10^3 cells/ml	42.7×10^3 cells/ml
NAGase activity	2.09 nmol min^{-1} ml^{-1}	1.48 nmol min^{-1} ml^{-1}
Metabolic status of the cow		
Urea	4.35 mmol/l	4.44 mmol/l
β-hydroxybutyrate	1.65 μmol/l	1.63 μmol/l

Fig. 10.1   Mean deviation (in $\log_{10}$) of selected milk constituents from the overall means in relation to cell count classes ($n = 9326$ samples).

when the cell count threshold of 100,000 cells/ml was exceeded, the concentration of all the aforementioned milk components moved out of the physiological ranges. Another study published in 2002 (Hamann, 2002b) confirmed these results and used the milk components NAGase, K, Cl, conductivity and lactose derived from 9326 quarter composite milk samples as parameters to describe the change in concentration related to different cell count classes. The mean values of these milk constituents were significantly different (t-test; $P < 0.001$) between the cell count classes (in 1000s) 0–50; 51–100; 101–200; 201–400 and > 400/ml milk. To summarize the results, Fig. 10.1 compares on a log scale the mean of each milk component for each somatic cell count range with the overall mean. The deviation from the overall mean of the milk components in each somatic cell count range is indicated. To improve understanding, the zero line acts as a reference for all milk components across the somatic cell count ranges.

It can be seen that all curves indicate a distinct slope change as the cell count level changed through the range of 100,000/ml. This confirms the assumption that the physiological norm will be about 100,000 cells/ml.

Milk composition and interdependence between quarters of the identical mammary gland
Variations in milk composition and milk yield between udder quarters have often been attributed to anatomical structure differences. Yet, some publications throughout the last 20 years have clearly shown interactions between udder quarters with regard to milk yield (Woolford, 1985; Hamann and Reichmuth, 1990), growth of the glandular tissue (Knight and Peaker, 1991), somatic cell count (Hamann and Gyodi, 1994) and milk composition (Hamann *et al.*, 1999).

Additional studies on cell count level, differential cell count, cell functions and NAGase activities (Hamann *et al.*, 2005; Merle *et al.*, 2007) have shown that healthy quarters (cell count < 100,000 cells/ml; no pathogens detectable) of healthy mammary glands (four healthy quarters) had a significantly ($P < 0.05$) lower cell count level compared to healthy quarters in mammary glands with at least one infected quarter. Moreover, in healthy quarters of healthy glands, the differential cell count pattern and the function were significantly different ($p < 0.05$) in comparison with the values in healthy quarters in subclinically diseased glands. These findings confirmed earlier results which showed that milk components were dependent on the udder health status of neighbouring quarters (Hamann *et al.*, 1999). Overall, it can be concluded that an infection in a single quarter (milk cell count > 100,000/ml; bacteriologically positive detection of pathogens) can change the milk composition in the other three healthy quarters in the direction of the milk of the mastitic quarter (Hamann *et al.*, 2005). Moreover, some unpublished data show that mastitis quarters will evoke a significantly increased level of Vitamin A in the milk of healthy neighbouring quarters compared to healthy quarters of healthy mammary glands (Mann, 2009). The mechanisms for this type of interaction between healthy and diseased quarters within the same mammary gland are not completely understood. Very probably there are immunological local (udder) and systemic reactions involved (Merle *et al.*, 2007).

Herd bulk milk cell count level and antibiotic residues
Somatic cell counts are used to predict the health status of mammary glands. It can be assumed that unphysiologically high cell counts indicate increased inflammation and, very often, intramammary infections. Therefore, the risk of mastitis is significantly higher in herds with cell counts of several hundreds of thousands of cells per ml compared to herds with a bulk milk cell count of less than 200,000 or 100,000 cells/ml. On average, it can be stated that with a higher cell count, also the treatment frequency of antibiotic application in dairy cows is higher. Based on this interaction (Leslie *et al.*, 1996; Ruegg, 2005) there is some information indicating a relationship between herd somatic cell count level and detection of antibiotic residues in milk. These residues occurred mainly by human failures (not regarding the withholding periods after antibiotic application) (Vassallo, 2005). In conclusion it can be summarized that effective mastitis control would also be the most effective way to minimize the risk of violative levels of antibiotic residues in milk.

10.2.2   Raw milk safety
Raw milk safety is one part of food hygiene in addition to food sanitation, food protection and food preservation. Raw milk safety is determined mainly by the hygiene conditions in the farm, including the milking equipment and the health status of the dairy cow. The first priority of raw milk safety is the risk of bacterial contamination, in so far as mastitis is of concern, too.

With increasing global trade in milk products and European trade in milk and milk products, nearly all countries have tried to maintain the principally excellent image of milk products. Especially during recent years a series of new food laws, regulations and standards have been established with the primary goal of improving the prevention of any potential impairment of consumer health due to milk consumption (consumer protection). For the European Community some examples are as follows:

- Regulation (EC) No. 178/2002 of the European Parliament and the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and defining procedures in matters of food safety. This is the so-called basic regulation that especially states that the producer (i.e. farmer, dairy farmer, etc.) has the responsibility for quality and safety of the products he or she is bringing to the market.
- Regulation (EC) No. 852/2004 of the European Parliament and the Council of 29 April 2004 on the hygiene of foodstuffs.
- Regulation (EC) No. 853/2004 of the European Parliament and the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.
- Regulation (EC) No. 854/2004 of the European Parliament and the Council of 29 April 2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption.

In the USA, the US PHS (United States Public Health Service), as a functional unit of the FDA (Food and Drug Administration), installed the Grade 'A' Pasteurized Milk Ordinance (2003 Revision) on 2 March 2004 (US FDA, 2004). One of the highest priorities of this Ordinance consists of assuring the safety of milk and milk products. The sanitation programme proposed covers all areas from production to the final consumer. Several other countries (e.g. China, Australia, African countries, etc.) are actively introducing and/or improving food standards, hygiene and safety of dairy products (IDF, 2008).

Other risks exist, such as residues of therapeutic (drugs) or preventive agents (teat sanitation, plant sanitation). It can be concluded that as long as the official withdrawal periods are applied, the delivered milk will be free of antibiotic residues in terms of the relevant regulations in a country. Every milking plant or even a simple bucket milking system needs to be carefully cleaned and disinfected after milking. The common phases applied to cleaning and disinfection consist of pre-rinsing (hot or cold water), main cleaning (hot or warm water detergent–disinfectant treatment) and post-rinsing (hot or cold water). Under such conditions the potential residue level of detergents and cleaning solutions is so little that there will be no influence on the results of inhibitory screening tests. Moreover, such potential traces are free of any risk to consumer health. In addition, in the EU, most of the chemical components (e.g. iodine and chlorine compounds) are listed in Annex II of the Council Regulation (EEC) No. 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medical products in foodstuffs of animal

origin. The list in Annex II gives the substances not subject to maximum residue levels. Therefore, potential residues from such teat disinfection measures can be regarded as not relevant to milk safety (Council Regulation 2377/90).

It can be concluded that several harmful bacteria could be regarded as potentially important for raw milk safety so that consumers of raw milk and/or raw milk products are not exposed to unavoidable health risks.

Raw milk may contain a whole host of pathogens, including the example microorganisms listed in Table 10.2 (Hamann, 1997). As far as mastitic organisms are concerned, mainly *S. aureus* and *Str. agalactiae* are derived from infected mammary glands. All other pathogens are very seldom associated with subclinical mastitis cases. Thus, the majority of contaminated raw milk samples result from environmental contamination. On occasion cows may carry several pathogens without exhibiting any clinical symptoms. Yet, such cows may excrete the pathogens via the faeces. As such, contamination cannot be excluded absolutely; there will be a certain residual risk for consumers of raw milk and raw milk products.

Although several studies have been published indicating that foodborne pathogens may be present in raw milk and raw milk products, people continue to drink raw milk. Some people believe that raw milk has a higher nutritional value than pasteurized milk (Hegarty *et al.*, 2002) or that the taste and/or convenience are better (Jayarao *et al.*, 2006). To eliminate the existing risk, the consumption of raw milk should be avoided. Based on public health concerns, several official institutions have recommended cooking or pasteurizing any milk before consumption (BGVV, 1995; US FDA, 2004). This has resulted in regulations for the USA that permit sales of raw milk for human consumption in 28 out of 50 US states (Realmilk, 2007).

The comparison between raw milk and pasteurized milk shows clearly that pasteurized milk is really no different from raw milk in nutritional value and milk composition, while at the same time being safer for human beings to drink.

Table 10.2 Causes of contamination of milk with human pathogenic microorganisms

Pathogens	Subclinical disease occurrence		Importance of contamination source	
	Cow	Udder	Disease	Environment
Staph. aureus	−	+	+	−
Str. agalactiae	−	+	+	−
C. jejuni	+	−	−	+
Y. enterocolitica	+	+	−	+
Salmonella spp.	+	+	−	+
E. coli	+	+	+	+
L. monocytogenes	+	+	−	+
C. burnetti	+	+	−	+
M. paratuberculosis	+	+	−	+

10.2.3   Milk yield

Healthy bovine mammary glands secrete milk mainly depending on breed, lactation stage and number, feeding, management systems, genetic influences and milking process-related factors, such as milking machine process, milking frequency and milking intervals. If a quarter suffers from mastitis, depending on the type (clinical or subclinical) and degree (peracute, acute, chronic) of the disease, a significant milk yield decrease in a range of 10% to more than 80% can be observed. The inflammatory process will also depend on the status of the individual cow's defence mechanisms. The extent of the somatic cell count increases as an indicator of the inflammation, and the magnitude of the secretory depression in terms of milk losses will be determined by the type of pathogen (e.g. minor or major causative agents).

Mastitis is an inflammation of one or more quarters of a mammary gland, almost always caused by different species of a broad variety of infecting pathogens. The milk secretion activity (kg of milk) is identical for both front quarters and also for both hind quarters (Woolford, 1985). This statement is valid so long as all quarters are healthy. Mastitis will change the situation in so far as the infected quarter will decrease the milk yield, but healthy neighbouring quarters will, at least to some extent, compensate for the yield loss of the infected quarters. Based on experimental data it can be concluded that if two quarters per udder are infected, the other two healthy quarters of adult cows are able to react with a compensatory yield increase of about half of the infection-induced yield loss (Woolford, 1985; Hamann and Reichmuth, 1990). Overall, it has been estimated that the mean loss of milk yield per cow for every 100,000 increase in bulk tank somatic cell count per ml milk will result in a milk loss between 0.18 and 0.50 litres per cow per day (Hamann and Heeschen, 1995). In contrast to some publications in recent years, it could be demonstrated that the milk yield per quarter is significantly ($p < 0.001$) influenced by a very low somatic cell count. The data indicated that the yield per udder quarter dropped significantly ($p < 0.001$) from 315 to 266 g/h/quarter if the somatic cell counts increased from <50,000 to 51,000–100,000 cells/ml milk (Halm, 2003). Therefore, it can be concluded that the strongest milk yield loss in infected udder quarters will happen at relatively low somatic cell count levels in a range of <200,000 cells/ml milk (Hamann et al., 2004).

Mastitis is, for the dairy farmer, a very relevant disease for economic reasons. Two aspects are relevant: in many countries, the milk payment systems indicate a milk price reduction when certain cell count thresholds in the herd bulk milk are exceeded; and secondly, the dairy farmer will see a milk yield loss in mastitis cows. The marked variation in different mastitis types in combination with mastitis-predisposing factors for a variety of diseases such as metabolic disorders, sterility, etc. interferes with the calculation of the economic losses by mastitis. So far, all publications, regardless of the type of model in use for the prediction of mastitis losses, give only a trend but no real values for mastitis losses.

Including newer data published by Berry et al. (2004) and Huijps and Hogeveen (2007), it is estimated that in high-yielding herds the economic loss

due to mastitis per cow in a dairy herd will be in the range of 200 euros/year (Hamann, 2008).

10.3   Causes of mastitis

The majority of mastitis cases are caused by different types of pathogens. In addition to traumatic lesions it is very common for pathogens to be transferred during milking (mainly cow-associated pathogens such as *S. aureus* and *S. agalactiae*) or during the intermilking interval (e.g. coliforms, *S. uberis*) to the area of the internal or external side of the teat canal orifice. Depending on the individual cow, systemic immunological or local defence mechanisms, the pathogens present may have a chance to get fixed to the epithelial surfaces. If they do get fixed, they can be called colonized pathogens.

10.3.1   Type of pathogens

All dairy cows are exposed to numerous microorganisms that can cause mastitis. Yet, the level of exposure shows a wide variation between cows and herds, depending on the conditions of hygiene in the cow environment, the hygiene management applied and the prevalence of mastitis in a particular herd. Therefore, it can be concluded that mastitis cannot be eradicated completely, but the rate of new infections can be maintained at a very low level by systematic monitoring of all mastitis cases and continuous application of mastitis control measures. Mastitis pathogens can be divided into three epidemiological groups: the contagious, cow-associated pathogens (e.g. *S. aureus*, *S. agalactiae*), the non-contagious, environment-associated bacteria (e.g. *S. uberis*, *E. coli*) and skin flora opportunists (e.g. coagulase-negative staphylococci, CNS). It is estimated that worldwide the majority of mastitis-causing pathogens belong to the group of contagious, cow-associated bacteria, e.g. *S. aureus* (Zecconi *et al.*, 2006). Yet, under very modern dairying conditions it may be that the majority of pathogens causing clinical mastitis belong to the group of environmental bacteria (Williamson, 2007).

10.3.2   Movement of infection

The contagious, cow-associated bacteria are primarily transmitted from infected to uninfected quarters in connection with the milking process. The most obvious way in which infection can spread from one cow to another is by mechanical transfer via contaminated teatcups. Movement of infection from teat to teat may happen if contaminated milk or milk droplets are transferred from one teat to another via the claw piece (Nyhan and Cowhig, 1967). Such contamination of the teat end can be caused by those vacuum fluctuations that are able to generate phenomena such as reverse flow, reverse spray and jet flow.

   Contamination with non-contagious, environment-associated pathogens will take place predominantly during the intermilking interval (Bramley, 1992). The

hygiene status of housing conditions (e.g. climate, wetness, dirty and contaminated bedding material) is the main contributor to the transmission of environment-associated bacteria. The epidemiology of the CNS is not well understood, but the most important time for transmission of these pathogens seems to be the dry period.

10.3.3   Invasion and infection

Under physiological conditions, the teat canal acts as an effective valve guarding the entrance of the teat cistern. The mechanical forces applied by the milking machine dilate the teat apex and cause the teat canal to unfold. Obviously, the effectiveness of the teat canal to prevent penetration of mastitis pathogens is influenced by the interaction between the milking system applied and the corresponding reaction of the teat canal characteristics (diameter, thickness of keratin layer, rate of cornification of the *Stratum corneum*) (Hamann, 1989).

It is generally accepted that most of the pathogens gain access to the mammary gland by invasion through the teat canal. Yet, the detailed mechanisms involved in the invasion process are still not completely understood. If pathogens penetrate into the mammary gland, the potential of the immunological defence mechanisms inside the udder decides whether an infection will be initiated.

10.3.4   Inflammation and susceptibility

Inflammation as the reaction to the recognized presence of pathogens is principally a fundamental, positive event. All direct and indirect defence systems of the cow try to eliminate or to destroy the invading pathogens. If such inflammatory changes inside the mammary gland (e.g. increased number of somatic cells, increased concentration of NAGase, etc.) are successful in eliminating the pathogens, the inflammatory changes will recover to a physiological level within weeks. If the pathogens survive, chronic mastitis, sometimes for life, will follow depending on the type of the causative agent.

A long list of potentially responsible factors have been discussed concerning susceptibility to mastitis. Very often it has been stressed that with increasing milk yields (>8000 kg milk per cow and lactation), the susceptibility to mastitis would also increase more or less automatically. Meanwhile, it could be shown that high-yielding cows exhibit lower susceptibility compared with low yielders (Müller and Weber, 2007; Rehage and Kaske, 2004). A very convincing study was performed in four field herds with a total of 1900 cows. The yield levels were grouped in steps of 1000 kg between 5000 and 12,000 kg per cow and lactation. The overall results showed that no significant differences between the milk yield categories could be found for number of treatments per cow and for different disease problems (e.g. lameness, metabolic disorder, mastitis, sterility). The conclusion was that high-yielding cows are not more often diseased and do not need more frequent therapeutic treatments. Yet, the requirements for

management (early detection of diseases, cow-related feeding, yield level-related feeding ratio and regime, continuous application of preventive measures) are much higher for high-yielding cows than for low yielders (Wangler and Sanftleben, 2007).

10.4   Mastitis control

Mastitis prevention should consist of two main aspects: reduction of number and type of pathogens gaining access to the mammary gland, and maintaining a physiological balance of the cow (homoeostasis) so that the cow is able to use efficiently her complete spectrum of defence mechanisms. Mastitis prevention has the goal of reaching and maintaining a low new infection rate. In addition to preventive measures, the elimination of existing mastitis infections (e.g. therapy, culling) is needed to reduce the new infection risk. The so-called 'five point plan', developed about 45 years ago in Reading, UK, is a very useful strategy to control contagious mastitis. This programme consists of the following steps: (i) teat dipping of all teats after every milking; (ii) prompt treatment of all clinical cases; (iii) dry cow therapy on all cows that remain in the herd; (iv) culling of chronically infected cows; and (v) correct maintenance of the milking machine and its function (IAH, 2006). However, bacteria that are spread via the environment cannot be controlled sufficiently by the 'five point plan'. Additional measures are needed to keep the bacterial contamination of the teat end low (e.g. careful cleaning of the teat end before milking; pre-milking teat disinfection; avoidance of organic bedding material and application of sand; application of internal teat sealants to minimize the new infection rate throughout the dry period). It has been shown also that an adequate application of dietary levels of vitamins and trace elements supports the reduction of new infections by environmental pathogens (Erskine, 1993).

Antibiotic therapy should be used in clinical cases to reduce the pain a cow suffers (welfare aspects) and to try to eliminate the intramammary pathogens (Hillerton, 1997). Chemotherapy is also recommended for fresh subclinical cases caused by contagious pathogens within the first 90 days of lactation (Zecconi, 2006). Concerning the CNS, it can be assumed that regular post-milking teat dipping and total dry cow therapy reduce effectively new CNS infections to a low level (Smith and Hogan, 2001).

No doubt, antibiotic therapy of mastitis is a relevant aspect of mastitis control. We should remember that an antibiotic therapy is nothing more than a supporting factor for the body's defences to kill or to eliminate the bacteria. As the potential of all the immunological body's defences is dependent on the cow's status (feeding, lactation stage, endogenous and exogenous stressors), it is of great importance to keep the cow in a homoeostatic state. Yet, the key to successful mastitis control is effective prevention.

An effective and economically acceptable mastitis prevention programme needs regular monitoring at least of key parameters (number of clinical cases,

treatments, cow composite milk cell count, herd bulk milk cell count). The trend of the herd bulk milk cell count is a useful indicator for the development of mastitis in a herd. Depending on continuous monitoring of such information, early detection of mastitis problems and the initiation of specific control measures are possible.

10.5   Future trends

Malnutrition can be characterized mainly as the lack of sufficient intake of energy, protein and micronutrients (e.g. Vitamin A, Fe, iodine). It is assumed that more than 800 million people are undernourished worldwide (World Bank, 2007). Since 1999, the average values of calorie consumption for all types of countries and the world in general indicate levels in the range or above the minimum requirement for human beings (2500 kcal/capita/day) (DGE, 2000). Yet, the situation is quite different if we consider the consumption of animal food. On average the mean consumption of animal food is three times higher (about 1000 kcal/capita/day) in industrialized countries than in other countries (FAO, 2002). It is estimated that the demand for such food (e.g. milk and meat) driven by economic growth will increase dramatically within the next few years (World Bank, 2007). The annual increase in milk production is estimated to be about 0.4% for industrialized countries compared with about 4% for developing countries. Therefore, despite the progress we have made in countries with a modern dairy industry (e.g. the US, Canada, Europe, etc.) concerning milk quality and milk safety, we have to support countries like China and India so that they can improve markedly their milk safety. One of the main problems may consist in the number of milk producers (more than 150 million in China and India) with an average herd size below three cows. We have to try to transfer stepwise the information on milk quality standards, milk safety and improvement of hygiene management at the production level. Only if we reach an acceptable standard for animal food in such countries, can we assume that the global trade in food will be successful in the future. Very probably, we have to develop for that purpose certain new procedures to increase food quality by the application of simple and effective hygiene measures.

10.6   Sources of further information and advice

International organizations such as the International Dairy Federation (IDF) in Brussels, the World Health Organization (WHO) as a special organization of the United Nations in Geneva, and the Food and Agriculture Organization (FAO) in Rome have plentiful information on worldwide milk production, milk consumption, standards of milk quality, milk payment schemes and structures of dairy farming and milk distribution systems. In many cases, several countries have special national milk marketing boards or organizations, which can provide

detailed information for a single country. Regulations, directives and standards for milk quality and milk safety are discussed and decided by different organizations such as the European Commission (EC) for the European Union, and the FDA for the USA. The Codex Alimentarius Commission in Rome, created in 1963 by FAO and WHO, has 155 member states. This commission's main task is to develop food standards and guidelines to ensure consumer health and fair international food trade. Moreover, the Codex Alimentarius coordinates all work for defining food standards by international governments and non-governmental institutions.

A global organization for mastitis control and milk quality, the NMC, in Verona, WI, USA, provides an international forum for exchange of information on mastitis and milk quality and safety. Several publications including books and proceedings of the annual meetings are available. Different universities and agricultural and veterinary departments perform research in the area of milk production, milk quality and safety. Actual results of such research studies can be obtained from publications in journals such as the *Journal of Dairy Science*, *Journal of Dairy Research* and *Milchwissenschaft* (Milk Science International).

10.7   References

ADR (2008), *Rinderproduktion in Deutschland 2007: Ausgabe 2008 der Arbeitsgemeinschaft Deutscher Rinderzüchter e.V.*, Bonn, ADR.

ALLISON J R D (1985), 'Antibiotic residues in milk', *Br Vet J*, 141, 121–124.

BARBANO D M (1999), 'Influence of mastitis on cheese manufacturing', in *Practical Guide for Control of Cheese Yields,* Brussels, International Dairy Federation, 19–27.

BERRY E A, HOGEVEEN H and HILLERTON J E (2004), 'Decision tree analysis to evaluate dry cow strategies', *J Dairy Res*, 71, 409–418.

BGVV (1995), 'Viele Lebensmittelinfektionen lassen sich vermeiden', press release of 15 May 1995, Berlin, BGVV.

BRAMLEY A J (1992), 'Milk hygiene and machine milking', in Bramley A J, Dodd F H, Mein G A and Bramley J A, *Machine Milking and Lactation*, Newbury, Berkshire, UK, Insight Books, 373–398.

BVL (2007), 'Bei Fleisch, Milch und Honig kaum Überschreitungen von Rückstandshöchstmengen', press release of 12 October 2007, Berlin, BVL.

COGAN T M (1972), 'Susceptibility of cheese and yoghurt starter bacteria to antibiotics', *App Micro*, 23, 960–965.

Council Directive 92/46/EEC of 16 June 1992 laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products. *Official Journal of the European Communities* L268 of 14 September 1992.

Council Regulation (EEC) No. 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medical products in foodstuffs of animal origin. *Official Journal of the European Communities* L224 of 18 August 1990.

DALTON J C (2006), 'Antibiotic residue prevention in milk and dairy beef', *Western Dairy News*, 4, W-79.

DEWDNEY J M and EDWARDS R G (1984), 'Penicillin hypersensitivity – is milk a significant hazard?: a review', *J Royal Soc Med*, 77, 866–877.

DGE (2000), *Referenzwerte für die Nährstoffzufuhr*, Frankfurt, Umschau Braus.

DIN, EN, ISO (1994), 'Normen zum Qualitätsmanagement und zur Qualitätssicherung/QM-Darlegung. Teil 1: Leitfaden zur Auswahl und Anwendung', DIN EN ISO 9000-1, Berlin, Harald Beuth.

DOGGWEILER R and HESS E (1983), 'Zellgehalt in der Milch ungeschädigter Euter', *Milchwissenschaft*, 38, 5–8.

DVG (1994), 'Leitlinen zur Bekämpfung der Mastitis des Rindes als Herdenproblem', Gießen, Deutsche veterinärmedizinsiche Gesellschaft e.V.

ERSKINE R J (1993), 'Nutrition and mastitis', *Food Anim Prac*, 9, 551–561.

FAO (2002), *World Agriculture: Towards 2015/2030*, Rome, Food and Agriculture Organization.

HALM H (2003), 'Zum Einfluss eines automatischen Melkverfahrens auf Milchmengenleistung und Milchinhaltsstoffe hochleistender DH-Kühe unter Berücksichtigung von Laktationsstadium und Eutergesundheit', Univ Vet Med, Hannover, Vet Med Diss.

HAMANN J (1989), *Maschineller Milchentzug und Mastitis*, Stuttgart, Enke.

HAMANN J (1997), 'Tiergesundheit und Milchqualität: Grundlagen des Verbraucherschutzes', in Rektor der Tierärztlichen Hochschule Hannover, *Forschung fürs Leben*, Trier, Druckerei Paulinus, 8–13.

HAMANN J (2002a), 'Milk quality and udder health in relation to modern milking technique', in Kaske M, Scholz H and Höltershinken M, *Recent Developments and Perspectives in Bovine Medicine,* Hildesheim, Hildesheimer Druck- und Verlags-GmbH, 334–345.

HAMANN J (2002b), 'Relationship between somatic cell count and milk composition', *IDF Bull*, 372, 56–59.

HAMANN J (2008), 'Zur Bekämpfung der bovinen Mastitis unter Praxisbedingungen', *Tieräztl Umschau*, 63, 643–650.

HAMANN J and GYODI P (1994), 'Effects on milk yield, somatic cell counts and milk conductivity of short-term non-milking of lactating quarters of cows', *J Dairy Res*, 61, 317–322.

HAMANN J and HEESCHEN W (1995), 'Bovine mastitis – significance for milk yield, composition and hygiene', in Martens H, *IXth Int Conf Prod Dis Farm Anim 1995*, Stuttgart, Enke, 303–314.

HAMANN J and REICHMUTH J (1990), 'Compensatory milk production within the bovine udder: effects of short-term non-milking of single quarters', *J Dairy Res*, 57, 17–22.

HAMANN J, GYODI P, KRÖMKER V and STAHLHUT-KLIPP H (1999), 'Physiological variation of milk components in bovine udder quarters with special regard to milking frequency', in Wensing T, *Production Diseases in Farm Animals*, Wageningen, Wageningen Pers, 313.

HAMANN J, NOGAI K, REDETZKY R, GRABOWSKI N T and HEIDE A (2002), 'Milk constituents as tools for mastitis detection', *Proc Satellite Symp Novel Aspects of Mastitis Therapy*, Boehringer Ingelheim, Ingelheim, Knecht Digital- and Printmedien GmbH, 18–23.

HAMANN J, REDETZKY R and GRABOWSKI N T (2004), 'Cow-side mastitis tests – possibilities and limitations', *Proc Natl Mastitis Council Annu Meet*, 60–77.

HAMANN J, SCHRÖDER A and MERLE R (2005), 'Differential cell count and interdependence of udder quarters', in Hogeveen H, *Mastitis in Dairy Production – Current*

Knowledge and Future Solutions, Wageningen, Wageningen Academic Publishers, 190–195.

HEGARTY H, O'SULLIVAN M B, BUCKLEY J and FOLEY-NOLAN C (2002), 'Continued raw milk consumption on farms: Why', *Commun Dis Public Health*, 5, 151–156.

HILLERTON J E (1997), 'Mastitis therapy is necessary for animal welfare', *IDF Bull*, 330, 4–5.

HUIJPS K and HOGEVEEN H (2007), 'The farmers cost of mastitis', *IDF Animal Health Newsletter*, 14–15.

IAH (2006), Institute for Animal Health, 'Disease facts – mastitis and milk quality', www.iah.bbstc,ac.uk/disease/mastitis.shtml.

IDF (1967), 'Definition of mastitis – Influence of cold-storage of raw milk on certain of its properties', *Bull 034/1997*, Brussels, International Dairy Federation.

IDF (2008), 'Standards, hygiene and food safety of dairy products: risk management, practical food safety management, predictive modelling, emerging issues', *Bull 430*, Brussels, International Dairy Federation.

JAYARAO B M, DONALDSON S C, STRALEY B A, SAWANT A A, HEDGE N V and BROWN J L (2006), 'A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania', *J Dairy Sci*, 89, 2451–2458.

KNIGHT C H and PEAKER M (1991), 'Mammary gland physiology', *Flem Vet J*, 62, Suppl 1, 33–42.

LANGFORD F M, WEARY D M and FISHER L (2003), 'Antibiotic resistance in gut bacteria from dairy calves: a dose response to a level of antibiotics fed in milk', *J Dairy Sci*, 86, 3963–3966.

LESLIE K E, GODKIN M A, SCHUKKEN Y H and SARGEANT J M (1996), 'Milk quality and mastitis control in Canada: progress and outlook', *Proc Natl Mastitis Council Annu Meet*, 19–30.

MANN U (2009), 'Einflüsse parenteral supplementierter Vitamine auf das biochemische Blut – und Milchprofil und die Entwicklung der Eutergesundheit hochleistender Milchkühe', Univ Vet Med, Hannover, Vet Med Diss (in press).

MERLE R, SCHRÖDER A and HAMANN J (2007), 'Cell function in the bovine mammary gland: a preliminary study on ínterdependence of healthy and infected udder quarters', *J Dairy Res*, 74, 174–179.

MITCHELL J M, GRIFFITHS M W, MCEWEN S A, MCNAB W B and YEE A J (1998), 'Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance', *J Food Prot*, 61, 742–756.

MOATS W (1999), 'The effect of processing on veterinary residues in foods', in Jackson L S, Knize M G and Morgan J N, *Impact of Processing on Food Safety,* New York, Plenum, 233–241.

MÜLLER K E and WEBER C (2007), 'Hochleistungssport? – Die Energiebelastung der Milchkuh', www.fu-berlin.de/presse/publikationen/fundiert/2007.

NYHAN J F and COWHIG M J (1967), 'Inadequate milking machine vacuum reserve and mastitis', *J Vet Res*, 44, 1433–1438.

REALMILK (2007), 'What's happening with real milk', www.realmilk.com/happening.html, p. 27.

Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the The European Food Safety Authority and laying down procedures in mattes of food safety. *Official Journal of the European Communities* L031 of 1 February 2002.

Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. *Official Journal of the European Communities* L139 of 30 April 2004.

Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. *Official Journal of the European Communities* L139 of 30 April 2004.

Regulation (EC) No. 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. *Official Journal of the European Communities* L139 of 30 April 2004.

REHAGE J and KASKE M (2004), 'Interactions between yield and production diseases in dairy cows', *Proc Soc Nutr Physiol*, 13, 177–182.

REICHMUTH J (1975), 'Somatic cell counting – interpretation of results', *IDF Bull*, 85, 93–109.

RUEGG P (2005), 'Relationship between bulk tank milk somatic cell count and antibiotic residues', *Proc Natl Mastitis Council Annu Meet*, 28–35.

RUEGG P L and TABONE T J (2000), 'The relationship between antibiotic residue violations and somatic cell counts in Wisconsin dairy herds', *J Dairy Sci*, 83, 2805–2809.

SMITH K L (2002), 'A discussion of normal and abnormal milk based on somatic cell count and clinical mastitis', *IDF Bull*, 372, 43–45.

SMITH K L and HOGAN J S (2001), 'The world of mastitis', in NMC and AABP, *2nd Int Symp on Mastitis and Milk Quality, Vancouver*, 1–12.

TOLLE A and HEESCHEN W (1975), 'Der aseptische Milchentug über implantierte Dauerkatheter als Modell einer pulsierungsfreien Melktechnik und als Grundlage zum Studium der spezifischen und unspezifischen Infektionsabwehr', *Sdrh Ber Ldw*, 190, 60–71.

TOLLE A, HEESCHEN W, REICHMUTH J and ZEIDLER H (1971), 'Counting of somatic cells in milk and possibilities of automation', *Dairy Sci Abstr*, 33, 875–879.

US FDA (2004), CFSAN/Office of Compliance, 'Grade 'A' pasteurized milk ordinance 2003 revision, www.cfsan.fda.gov/~ear/pmo03toc.html.

VASSALO R (2005), 'Preventing antibiotic residues in milk', Farmnote No. 47/2005, Department of Agriculture, Government of Western Australia.

WANGLER A and SANFTLEBEN P (2007), 'Behandlungshäufigkeit bei Milchkühen in Praxisbetrieben in Abhängigkeit von der Milchleistung', *Tierärztl Praxis*, 35 G, 408–413.

WIESNER E and RIBBECK R (2000), *Lexikon der Veterinärmedizin*, Stuttgart, Enke.

WILLIAMSON J (2007), 'Environmental mastitis control', *IDF Bull*, 416, 41–47.

WOOLFORD M W (1985), 'The relationship between mastitis and milk yield', *Kieler Milchw ForschBer*, 37, 224–232.

WORLD BANK (2007), *World Development Report 2008 – Agriculture for Development*, Washington, DC, World Bank.

ZECCONI A (2006), 'Contagious mastitis control', *IDF Bull*, 416, 34–40.

ZECCONI A, CALVINHO L and FOX L (2006), '*Staphylococcus aureus* intramammary infections', *IDF Bull*, 408, 1–36.

11

Quality assurance schemes on the dairy farm

O. Cerf, Alfort Veterinary School, France, J.-M. Gautier and P. Parguel, Livestock Institute, France

Abstract: The hierarchy of international and national standards and guidelines that dairy farmers can apply to ensure milk quality and safety is presented. At the moment, ISO standards on quality management (ISO 9000) and food safety management (ISO 22000) are too exacting for the average farm. Farmers will continue for some time to achieve good practices as described in broad terms by the Codex Alimentarius Commission, the Food and Agriculture Organization of the United Nations, the International Dairy Federation and the World Organization for Animal Health and adapted in more practical and specific terms by national governments and/or farmer associations.

Key words: quality assurance, food safety, good hygienic practice, HACCP, dairy farm.

11.1 Introduction

The concepts of quality assurance (QA) have been developed fairly recently to cope with global economic growth, the diversification of industrial companies and the new demands of clients along the production chain up to the final consumer. The first quality control handbook seems to date back to 1951 (Juran, 1951), and the 'zero default' conceptualization to 1967 (Crosby, 1967). W.E. Deming in the USA (Deming Cycle Plan – Do-CheckAct) and K. Ishikawa in Japan (Ishikawa Diagram and the 6 M's, 8 P's and 4 S's) made significant contributions to the popularization of QA concepts (Deming, 1982; Ishikawa,

Table 11.1   Texts related to QA in dairy farming, listed in increasing order of specificity

ISO 9000	Quality management systems
ISO/IEC 17021	Requirements for bodies providing audit and certification of management systems
ISO 22000	Food safety management systems
ISO/TS 22003	Requirements for bodies providing audit and certification of food safety management systems
Codex Alimentarius	General principles of food hygiene
Codex Alimentarius	Code of hygienic practice for milk and milk products
IDF/FAO	Guide to good farming practice
OIE/FAO	Guide to good farming practices for animal production food safety
IDF	Guide to good animal welfare in dairy production
IDF	Code of good hygienic practices for milking with automatic milking systems
Governmental organizations, associations of farmers, etc.	Guides to good practice, recommendations, charters, etc.

1983). The food industry, including the dairy industry, embarked quickly in the QA movement. In France, for example, encouragement came from the Ministry of Agriculture (Creyssel, 1987; Mainguy, 1989).

In this chapter we will present briefly the standards of the International Organization for Standardization (ISO) that provide the general frame of QA, and those that focus on the management of food safety. We will then describe, among the standards of the Codex Alimentarius Commission (the Joint Food Standards Programme of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO)), those that deal with the hygiene aspects of the quality of foods. One of them is devoted to milk and milk products, including the primary production at the farm. We will end with the codes of practice with relevance to dairy farming published by FAO and the International Dairy Federation (IDF) and mention a few national schemes. The hierarchy of these standards is presented in Table 11.1.

11.2   Standards of the International Organization for Standardization

To harmonize the way quality assurance is understood and implemented over the world, the first international standards were drafted from 1987. The definition of quality assurance (QA) given in 1994 by ISO was: '*All the planned and systematic activities implemented within the quality system, and demonstrated as needed, to provide adequate confidence that an entity will fulfill requirements for quality*' (ISO, 1994). In the context of the present chapter, '*entity*' would

designate a dairy farm. Yet as discussed below, no dairy farm so far has applied the QA principles except in an experimental context.

QA is achieved through a quality management system, that is a '*system where an organization needs to demonstrate its ability to consistently provide product that meets customer and applicable statutory and regulatory requirements, and aims to enhance customer satisfaction through the effective application of the system, including processes for continual improvement of the system and the assurance of conformity to customer and applicable statutory and regulatory requirements*' (ISO, 2008).

The application of the standards since 1987 has led to their constant evolution. In 1987, the ISO norms described quality as compliance to a set of specified requirements. The 1994 version introduced the notion of satisfaction of customer expectations. The 2000 version added the continuous process improvement, and substituted '*quality management*' for '*quality assurance*'. The 2008 version brought some wording improvements.

To profit from implementing a quality management system and win its customers' trust the entity needs to be certified by a third party. The certification process by '*bodies providing audit and certification of management systems*' is also standardized (ISO/IEC, 2005, 2006).

A first attempt by ISO to unite the QA and the HACCP concepts of quality management with the Codex Alimentarius approach of good hygienic practices and application of HACCP principles (see below) was made in the '*Guidelines on the application of ISO 9001:2000 for the food and drink industry*' (ISO, 2001). It was partly unsuccessful. The second attempt resulted in a set of standards, ISO 22000, 22003 and 22005, which deal specifically with '*Food Safety Management Systems*' and their certification (ISO, 2005a, 2005b, 2007). We will come back later to these.

11.3   Standards of the Codex Alimentarius Commission

The '*Recommended International Code of Practice – General Principles of Food Hygiene*' was first published in 1969 and was later augmented with the HACCP system (CAC, 2003). According to their introduction, '*These General Principles lay a firm foundation for ensuring food hygiene and should be used in conjunction with each specific code of hygienic practice, where appropriate, and the guidelines on microbiological criteria. The document follows the food chain from primary production through to final consumption, highlighting the key hygiene controls at each stage. It recommends a HACCP-based approach wherever possible to enhance food safety*'. The above sentences convey two recommendations: (i) that good hygienic practices are implemented first, and then enhanced through a HACCP-based approach; (ii) the application of a HACCP-based approach is not possible everywhere.

A specific Code of Hygienic Practice for Milk and Milk Products could not be adopted before 2004, when an agreement was eventually reached on how to

accommodate the widely different needs of countries where there is a majority of smallholder dairy farms,[1] and of industrialized countries including those where some raw milk products are allowed (CAC, 2004).

11.3.1   General principles of food hygiene

The General Principles of Food Hygiene are presented in very broad terms. As the title indicates, they are nothing more than a list of principles, without guidance on application. For example, only one page is devoted to primary production, introduced by:

> '**OBJECTIVES**: *Primary production should be managed in a way that ensures that food is safe and suitable for its intended use. Where necessary, this will include:*
>
> – *avoiding the use of areas where the environment poses a threat to the safety of food;*
> – *controlling contaminants, pests and diseases of animals and plants in such a way as not to pose a threat to food safety;*
> – *adopting practices and measures to ensure food is produced under appropriately hygienic conditions.*
>
> **RATIONALE:** *To reduce the likelihood of introducing a hazard which may adversely affect the safety of food, or its suitability for consumption, at later stages of the food chain.*'

11.3.2   Code of Hygienic Practice for Milk and Milk Products

The Code of Hygienic Practice for Milk and Milk Products is more helpful despite the fact that it is also a list of principles rather than providing practical guidance. The outline of the '*Section 3 Primary Production*' (3 pages) is given in Fig. 11.1. Emphasis is put on the following points:

- Environmental hygiene, notably of water used for milking
- Milk production in hygienically designed stables and milking parlours
- Animal health
- Hygienic feeding, pest control, good use of veterinary medicines
- Hygienic handling, storage and transport of milk
- Record keeping.

An important caveat is given in '*Section 2.3 Overarching principles applying to the production, processing and handling of all milk and milk products*':

---

1. In the context of the Codex Code of Hygienic Practice for Milk and Milk Products, '*smallholder dairy farm*' refers to '*farms where the number of animals per farmer or per herd usually does not exceed 10, milking machines are not generally used, milk is not chilled at the producer's level and/or the milk is transported in cans*'.

3.1 ENVIRONMENTAL HYGIENE

3.2 HYGIENIC PRODUCTION OF MILK

  3.2.1 Areas and premises for milk production
  3.2.2 Animal health
  3.2.3 General hygiene practice

      3.2.3.1 Feeding
      3.2.3.2 Pest control
      3.2.3.3 Veterinary drugs

  3.2.4 Hygienic milking

3.3 HANDLING, STORAGE AND TRANSPORT OF MILK

  3.3.1 Milking equipment
  3.3.2 Storage equipment
  3.3.3 Premises for, and storage of, milk and milking-related equipment
  3.3.4 Collection, transport and delivery procedures and equipment

3.4 DOCUMENTATION AND RECORD KEEPING

Fig. 11.1   Outline of 'Section 3 – Primary Production' of the Codex Alimentarius *Code of Hygienic Practice for Milk and Milk Products*.

'*Wherever appropriate, hygienic practices for milk and milk products should be implemented within the context of HACCP as described in the Annex to the* Recommended International Code of Practice – General Principles of Food Hygiene. *This principle is presented with the recognition that there are limitations to the full application of HACCP principles at the primary production level. In the case where HACCP cannot be implemented at the farm level, good hygienic practices, good agricultural practices and good veterinary practices should be followed.*'

Indeed Principle 2 of the HACCP system, viz. '*Determine the Critical Control Points*', does not apply in cases where there is no Critical Control Point (CCP), which is '*A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level*'. Principle 3 of the HACCP system states: '*Critical limits must be specified and validated for each Critical Control Point*'. A 'critical limit' is '*a criterion which separates acceptability from unacceptability*'. At the primary production level, there are no control measures for which a critical limit can be specified, hence there can be no CCP. Nevertheless, if control measures are essential they have to be implemented, ISO 22000 names them '*Operational Prerequisite Programs (oPRP)*' (ISO, 2005b). The definition of oPRP is a great achievement of ISO 22000 that adds flexibility and applicability to the Codex Alimentarius approach, for example at the dairy farm level. Yet because this norm is recent, all its advantages are still not fully acknowledged, and the associated costs of application retard its uptake.

11.4 Guides of the Food and Agriculture Organization

Together with the International Dairy Federation, the Food and Agriculture Organization of the United Nations (FAO) published a '*Guide to Good Dairy Farming Practice*' (IDF-FAO, 2004). It is intended to be used within a food safety and quality assurance management system, and can be considered a guide to 'prerequisite programs' (PRP) as defined in ISO 22000. The PRPs comprise good hygienic practice as well as good agricultural practice, good veterinary practice, etc. The IDF-FAO guide has the following outline:

- Animal health
- Milking hygiene
- Animal feeding and water
- Animal welfare
- Environment.

Together with the World Organization for Animal Health (OIE), FAO then published a '*Guide to Good Farming Practices for Animal Production Food Safety*' that aims at helping to adopt a more comprehensive approach to the control of animal health and food safety hazards at the farm level (OIE-FAO, 2008).

Again these two guides are generic, as they list principles without recommendations on their practical implementation. Amazingly the OIE-FAO guide does not give any hazard name, whether for animal or human health. The contents are:

- Farm management: record keeping, animal identification, hygiene and disease prevention (illnesses in relation to the environment), training of farmers
- Management of animal health (including physical integrity of animals): addressing biohazards and physical hazards
- Veterinary medicines and biologicals, complying with veterinary prescription and restriction for use in livestock
- Addressing biological, chemical and physical hazards for animal feeding and watering, for environment and infrastructure, for animal product handling.

11.5 Guides of the International Dairy Federation

To provide practical guidance, IDF published the documents presented below. Recent developments of good dairy farming practices were discussed at a recent International Dairy Congress (IDF, 2007).

11.5.1 Code of Good Hygienic Practices for Milking with Automatic Milking Systems (IDF, 2004)

Considering the influence of milking machines on milk quality and hygiene, and the increasing number of milking robots, IDF has updated its previous recommendations (Reinemann *et al.*, 2003). The code insists on changes in livestock

management, animal health monitoring, training of farmers, record keeping, milking robot design, individual follow-up of animals and monitoring and diversion of abnormal milks. It is a useful complement to the IDF-FAO Guide (IDF-FAO, 2004).

11.5.2   Guide to Good Animal Welfare in Dairy Production (IDF, 2008)

This guide is a further complement to the IDF-FAO guide (IDF-FAO, 2004). Animal welfare is not something to disregard, considering its influence on animal health and production (McInerney, 2004). Recommendations are given on:

- Stockmanship: training on the care of animals, knowledge of animal appearance and behaviour, compassion, avoidance of practices that cause suffering
- Feed and water quality and quantity
- Physical environment (milking parlours, handling yards, feedlots and yards for holding animals, housing, shade and shelters in outside position)
- Husbandry practices minimizing pain and risk of injury and distress, provision of a comfortable milking system and optimum transport conditions
- Health management, notably as regards lameness, mastitis, acidosis of dairy cows, diarrhoea, anaemia of calves, respiratory disease, etc.

11.6   National and specific guides

To provide concrete advice, national bodies have published their own guides aimed at food safety. According to a recent Regulation enforced in the European Union, '*Member States shall encourage the development of national guides to good practice for hygiene and for the application of HACCP principles*' (EC, 2004). Several national bodies have also implemented audit and certification schemes.

Some guides are written and published by governmental organizations. An example is the *Milk Hygiene on the Dairy Farm Guide – Northern Ireland*, published by the UK Food Standards Agency (FSA, 2008). Elsewhere guides are published by professional organizations. An example is the Canadian Quality Milk (CQM) programme of the Dairy Farmers of Canada (DFC, 2003). The French Arilait Guide (ARILAIT, 1997) is a kind of application manual, whereas the Charter of Good Practice for Livestock Farming (ARILAIT, 1997; CNE, 2007) is more of a guide. In countries of the European Union, guides written by a professional association can be assessed by the administration (EC, 2004). An example is the French guide of good hygienic practices for the making of milk products and cheeses at the farm, that includes good hygienic practices for the milk production itself (DTEQ, 2004), in addition to those in the Mutual Code of Goat Breeding (ANICAP, 2005).

Certification is the core of programmes by the Dutch Chain Quality of the Milk Foundation (KKM) (WCFS, 2000), the UK National Dairy Farm Assured

Scheme (ADF, 2008), Dairy Australia (Dairy Australia, 2007) and the external audit scheme for the French Charter of Good Practice for Livestock Farming (S. Picard, personal communication), and certification is also included in the above-cited Canadian programme (DFC, 2003). Certification can also be requested by retailers (EUREPGAP, 2008).

As regards livestock, QA has been adopted so far for limited applications aiming at quality in the broad sense of the term. Examples can be found in Germany (the QS-System for Food, Agriculture and Husbandry: QS, 2008), France (the HECTOR Program of the Livestock Institute, for cattle: Catalon and Carotte, 2004), etc. QA management of quality and environment in order to control the relations between farmers and cooperatives is another example of standardization (AFNOR, 2004a, 2004b).

11.7 Conclusions

The application of QA management principles in dairy farms requires a frame of reference according to the ISO 9000 standards, and a certification system. As regards food safety the standards ISO 22000 and 22003 provide the basis for the frame and for the management approach. Codex, FAO, OIE and IDF publications provide additional information about the frame. Yet more help is needed for the farmer to implement these good hygienic practices. It is provided by national organizations, governmental or professional, under the form of guides to good hygienic practices and application of the HACCP principles. It is recognized that, in most dairy farms, even if its principles are always applicable, the full HACCP system itself cannot be developed, as in general there is no critical control point (CCP). Identified hazards that are not reduced to an acceptable level by the sole application of good hygienic practices can, nevertheless, be controlled by one or a combination of operational prerequisite programmes (oPRP).

To our knowledge, the ISO 9000 standard has not been fully applied in dairy farms, and an associated certification system according to ISO-IEC 17021 does not exist. There is a long way to go before the QA management principles are fully applied and certified. If the ISO 22000 system gains more acceptance in the dairy industry, then milk production could be considered a step of the process. The farm could become a part of the 'entity' as defined in ISO 9000, and the dairy industry could take the responsibility for the formalization and the costs, including those of certification.

In the meantime, improvement of quality at the dairy farm level will continue to be attained through the fulfilment of the good practices described in innumerable national and international documents. Presumably most dairy farms cannot and will not be able to implement intense, time-consuming and expensive QA management systems that were created to satisfy the needs of manufacturing enterprises.

11.8   References

ADF (2008) *Standards and guidelines for assessment*. Ed. 3.2 (http://www.ndfas.org.uk/ accessed 1 February 2010), Dumfries, UK, Assured Dairy Farms.

AFNOR (2004a) *Système de management de la qualité de la production agricole – Modèle pour la maîtrise des engagements réciproques entre les producteurs et une structure organisée de la production agricole pour répondre aux attentes clients – NF V01-005*, May 2004, La Plaine Saint-Denis, France, Association Française de Normalisation.

AFNOR (2004b) *Système de management de la qualité et de l'environnement de la production agricole – Modèle pour la maîtrise des engagements réciproques entre les producteurs et une structure organisée de production agricole pour répondre aux attentes des clients et parties intéressées – NF V01-007*, December 2004, La Plaine Saint-Denis, France, Association Française de Normalisation.

ANICAP (2005) *Code mutuel de bonnes pratiques en élevage caprin*, Paris, Association Nationale Interprofessionnelle Caprine.

ARILAIT (1997) *Le HACCP et la filière lait, volume 2: le HACCP en production laitière*, Paris, Arilait-Recherche, FNPL.

CAC (2003) *Hazard Analysis and Critical Control Point (HACCP) System and Guidelines for its Application – Annex to CAC/RCP 1-1969 (Rev. 4 – 2003)*, Rome, Codex Alimentarius Commission.

CAC (2004) *Code of Hygienic Practice for Milk and Milk Products*, Rome, Codex Alimentarius Commission.

CATALON, L. and CAROTTE, G. (2004) Programme HECTOR: Adaptation et développement des principes et outils du Management Total de la Qualité (TQM) en élevage laitier (http://www.inst-elevage.asso.fr/html1/IMG/pdf/13._Programme_Hector.pdf, accessed 1 February 2010). Paris, Institut de l'Elevage.

CNE (2007) Charte des bonnes pratiques d'élevage (http://www.web-agri.fr/ dossier_special/space-2007/default.asp?idDoss=76&id=42009, accessed 1 February 2010). Paris, Confédération Nationale de l'Elevage.

CREYSSEL, P. (1987) *Conclusions de la commission d'experts sur la modernisation du droit de l'alimentation*, Paris, Ministère de l'Agriculture.

CROSBY, P. B. (1967) *Cutting the Cost of Quality: The Defect Prevention Workbook for Managers*, Boston, MA, Industrial Education Institute.

DAIRY AUSTRALIA (2007) On farm quality assurance – The dairy industry's approach to supply chain management (http://www.dairyaustralia.com.au/en/Responsible-Dairying/On-Farm-Quality-Assurance.aspx, accessed 17 December 2008). Southbank, Victoria, Australia, Dairy Australia.

DEMING, W. E. (1982) *Quality, Productivity, and Competitive Position*, Cambridge, MA, Massachusetts Institute of Technology Center for Advanced Engineering Study.

DFC (2003) *Canadian Quality Milk – On-Farm Food Safety Program Reference Manual* (http://www.dairyinfo.gc.ca/pdf/referencemanual.pdf, accessed 1 February 2010). Ottawa, Canada, Dairy Farmers of Canada.

DTEQ (2004) *Guide de bonnes pratiques d'hygiène pour les fabrications de produits laitiers et fromages fermiers*, Paris, Département Techniques d'Elevage et Qualité – Institut de l'Elevage/Technipel.

EC (2004) Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. *Official Journal of the European Communities*, L226, 3–21.

EUREPGAP (2008) *Integrated Farm Assurance* (http://www.eurepgap.org/farm/Languages/ English/documents.html, accessed 1 February 2010), Cologne, Germany, EUREPGAP.

FSA (2008) *Milk Hygiene on the Dairy Farm Guide – Northern Ireland* (http:// www.food.gov.uk/foodindustry/guidancenotes/hygguid/fhlguidance/milkfarmhygni, accessed 1 February 2010). London, Food Standards Agency.

IDF-FAO (2004) *Guide to Good Dairy Farming Practice*, Brussels, International Dairy Federation.

IDF (2004) Code of good hygienic practices for milking with automatic milking systems. *Bulletin of the International Dairy Federation*, 386, 43–49.

IDF (2007) Good dairy farming practices related to primary production of milk and farm management. *Bulletin of the International Dairy Federation*, 418, 3–42.

IDF (2008) *Guide to Good Animal Welfare in Dairy Production*, Brussels, International Dairy Federation.

ISHIKAWA, K. (1983) *Guide to Quality Control*, Hong Kong, Nordica International.

ISO (1994) *Quality management and quality assurance – Vocabulary*, ISO 8402:1994, Geneva, International Organization for Standardization.

ISO (2001) *Guidelines on the application of ISO 9001:2000 for the food and drink industry*, ISO 15161:2001, Geneva, International Organization for Standardization.

ISO (2005a) *Food safety management systems – Guidance on the application of ISO 22000:2005*, ISO/TS 22004:2005, Geneva, International Organization for Standardization.

ISO (2005b) *Food safety management systems – Requirements for any organization in the food chain*, ISO 22000:2005, Geneva, International Organization for Standardization.

ISO (2007) *Food safety management systems – Requirements for bodies providing audit and certification of food safety management systems*, ISO/TS 22003:2007, Geneva, International Organization for Standardization.

ISO (2008) *Quality management systems – Requirements*, ISO 9001:2008, Geneva, International Organization for Standardization.

ISO/IEC (2005) *Conformity assessment – Guidance on the use of an organization's quality management system in product certification*, ISO/IEC Guide 53:2005, Geneva, International Organization for Standardization

ISO/IEC (2006) *Conformity assessment – Requirements for bodies providing audit and certification of management systems*, ISO/IEC 17021:2006, Geneva, International Organization of Standardization

JURAN, J. M. (1951) *Quality-control Handbook*, Columbus, OH, McGraw-Hill.

MAINGUY, P. (1989) *La qualité dans le domaine agro-alimentaire*, Paris, Rapport de mission, Ministère de l'Agriculture et de la Forêt, Secrétariat à la Consommation.

MCINERNEY, J. P. (2004) *Animal Welfare, Economics and Policy. Report for Defra*, London, UK Department for Environment, Food and Rural Affairs.

OIE-FAO (2008) OIE-FAO guide to good farming practices for animal production food safety. *Bulletin of the OIE*, 2008-3, 5–12.

QS (2008) QS – Ihr Prüfsystem für Lebensmittel (http://www.q-s-info.de/, accessed 1 February 2010). Bonn, QS Qualität und Sicherheit GmbH.

REINEMANN, D. J., WOLTERS, G. M. V. H., BILLON, P., LIND, O. and RASMUSSEN, M. D. (2003) Review of practices for cleaning and sanitation of milking machines – Measuring vacuum in milking machines. *Bulletin of the IDF*, 381, 3–21.

WCFS (2000) Dutch dairy farming and dairy products (http://www.netherlands-embassy.org/files/pdf/dairy.pdf, accessed 17 December 2008). Wageningen, Netherlands, Wageningen Centre for Food Sciences.

12

Improving pasteurised and extended shelf-life milk

M. Lewis, University of Reading, UK

Abstract: This chapter focuses on the factors affecting the safety and quality of pasteurised milk for consumption and for conversion into products. It reviews the history of pasteurisation and compares what was known about it over sixty years ago, when batch processing was still commonplace and HTST processing was in its infancy to what is happening in current times. Today, HTST pasteurisers, producing pasteurised milk for the liquid market are operating in excess of 50,000 litres per hour for over 20 hours per day. If this milk is packaged into 1 litre containers, strategies are required to ensure that every one of these million containers is safe for consumption. In terms of removing pathogens and ensuring a long shelf-life, it is crucial to eliminate post pasteurisation contamination (ppc) and to ensure that the required pasteurisation times and temperatures are achieved. It is also important to ensure that raw milk destined for pasteurisation is of a consistently high quality. The temperature at which pasteurised milk is stored will also have a pronounced effect, with keeping quality increasing significantly as storage temperature is reduced from 8 to 2ºC. It is also important to avoid over processing, as this will inactivate lactoperoxidase and give rise to cooked milk flavours.

Key words: post pasteurisation contamination, pathogens, keeping quality, alkaline phosphatase, lactoperoxidase.

12.1   Introduction

Pasteurisation was first practised on wine prior to 1857 and slightly later on beer. In terms of milk processing, the first stage in the history of pasteurisation between 1857 and the end of the nineteenth century might well be called the

medical stage, as the main history in heat-treating milk came chiefly from the medical profession interested in infant feeding. The first commercial positive holder pasteurisation system for milk was introduced in Germany in 1895 and in the USA in 1907. As early as 1895, th requirements for an effective pasteurisation process were well recognised: 'we know that this process [pasteurisation] if properly carried out will destroy all disease germs' and 'a thoroughly satisfactory product can only be secured where a definite quantity of milk is heated for a definite period of time at a definite temperature. Then too, an apparatus to be efficient must be arranged so that the milk will be uniformly heated throughout the whole mass. Only when all particles of milk are actually raised to the proper temperature for the requisite length of time is the pasteurisation process complete.' This is still the main guiding principle underpinning current heat treatment regulations for ensuring a successful pasteurisation process, alongside ensuring that the product does not become contaminated after the heating process has been completed.

Since that time there have been many advances. Pasteurisation of milk is now readily accepted, although it did meet with resistance when first introduced, and still continues to do so from devotees of raw milk. The original objections to pasteurisation were summarised well by Satin (1996) and are presented in Table 12.1. He also pointed out at that time that many of these objectives are similar to those being encountered against food irradiation, as an alternative strategy for removing pathogens from food. Such resistance is also encountered in some quarters, for example by artisan cheesemakers. In May 2008, I noticed an obituary for Lucy Appleby, a cheesemaker who fought against pasteurisation and the supermarkets and was at last vindicated, selling a ton and a half a week (*Daily Telegraph*, 3 May 2008). She founded the Specialist Cheesemakers Association, a lobby group for the preservation of unpasteurised cheese. Appleby's now sells cheeses to Harrods and to Fortnum and Mason, along with exports to the USA, Singapore and the Caribbean. However, it was curious that, on my last inspection of their website, there was no mention that their cheese was made from raw milk.

Pasteurisation is now mostly performed as a continuous process, which is known as the high temperature, short time (HTST) process. This allows it to benefit from economies of scale. The capacities of modern HTST units can be up to 50,000 litres per hour and these units operate at high regeneration efficiencies (>95%) and are capable of long run times of up to 20 h before cleaning is required. This fits in well with currently important issues such as sustainability and improving the carbon footprint. In this context, however, pasteurised milk does require refrigeration to ensure a long shelf-life, which incurs additional energy requirements.

There are now alternative processes which strive to compete with thermal pasteurisation, such as irradiation, ultra-high pressures, and pulsed electric and magnetic fields. They have been reviewed by Barbosa-Canovas *et al.* (1998), Sun (2006) and, most recently specifically for milk, by Villamiel *et al.* (2009). Irradiation also offers great potential, particularly for inactivating pathogens in

Table 12.1   Some original objections to pasteurisation

(A) Sanitation
 1.   Pasteurization may be used to mask low-quality milk.
 2.   Heat destroys great numbers of bacteria in milk and thus conceals the evidence of dirt.
 3.   Pasteurization promotes carelessness and discourages the efforts to produce clean milk.
 4.   Pasteurization would remove the incentive for producers to deliver clean milk.
 5.   Pasteurization is an excuse for the sale of dirty milk.

(B) Physical and Bacteriological Quality
 1.   Pasteurization influences the composition of milk.
 2.   Pasteurization destroys the healthy lactic acid bacteria in milk, and pasteurized milk goes putrid instead of sour.
 3.   Pasteurization favors the growth of bacteria in milk.
 4.   Pasteurization destroys beneficent enzymes, antibodies, and hormones, and takes the "life" out of milk.

(C) Economics
 1.   Pasteurization legalizes the right to sell stale milk.
 2.   Pasteurization is not necessary in a country where milk goes directly and promptly from producer to consumer.
 3.   Pasteurization will increase the price of milk.
 4.   There are always some people who "demand raw milk."
 5.   If pasteurization is required, many small raw milk dealers will either have to go to the expense of buying pasteurizing apparatus or go out of business.

(D) Nutrition
 1.   Pasteurization impairs the flavor of milk.
 2.   Pasteurization significantly lowers the nutritive value of milk.
 3.   Children and invalids thrive better on raw milk.
 4.   Infants do not develop well on pasteurized milk.
 5.   Raw milk is better than no milk.

(E) Public Health and Safety
 1.   Pasteurization fails to destroy bacterial toxins in milk.
 2.   Imperfectly pasteurized milk is worse than raw milk.
 3.   Pasteurization, by eliminating tuberculosis of bovine origin in early life, would lead to an increase in pulmonary tuberculosis in adult life.
 4.   Pasteurization is unnecessary, because raw milk does not give rise to tuberculosis.
 5.   Pasteurization gives rise to a false sense of security.
 6.   It is wrong to interfere in any way with Nature's perfect food.
 7.   Pasteurization would lead to an increase in infant mortality.

Source: Reprinted from M. Satin, *Food Irradiation – A Guidebook*, p. 35, with permission from Technomic Publishing Co., Inc., copyright 1996.

solid foods such as poultry and seafood, but it still meets consumer resistance and it is possible that it will induce off-flavours in the fatty phase of milk. However, the major problem all these novel techniques face in gaining widespread acceptance is that thermal processes are so firmly established and capable of producing foods that are safe and of a high quality and nutritional value, in large volumes at very low processing costs. Some advances are being made, and

fruit juices subject to ultra-high pressures are now commercially available, at a cost. However, to date, these processes cannot compete in terms of scales of operation, long processing runs, energy efficiency and minimising waste, which, for HTST pasteurisation, is only produced at changeovers at the start and end of the process.

The description of pasteurisation given by the *IDF Bulletin* (1986) still remains appropriate. It is defined as a process applied with the aim of avoiding public health hazards arising from pathogenic microorganisms associated with milk, by heat treatment which is consistent with minimal chemical, physical and organoleptic changes in the product. This implies that pasteurised milk is little different from raw milk in terms of its sensory characteristics and nutrient content, although this would probably be contested by those devotees of raw milk. As well as *Mycobacterium tuberculosis*, Codex Alimentarius (2003) additionally mentions inactivation of *Coxiella burnetti*: 'As it is the most heat-resistant non-sporulating pathogen likely to be present in milk, pasteurisation is designed to achieve at least a 5 log reduction of *C. burnetti* in whole milk.' Thus, it will also result in a substantial reduction in populations of most pathogens that might be present in raw milk.

12.2   History of pasteurisation of milk

In order to chart the developments in the practice of pasteurisation, it is interesting to look at what was known about pasteurisation between 50 and 60 years ago, by reference to Cronshaw (1947) and Davis (1955). These sources still remain worth consulting.

Half way through the twentieth century (around 1950), batch processing was still widely used, but the principles of HTST processes were well established and continuous pasteurisers were available and were processing on average just under 10,000 litres per hour. As mentioned earlier, it was recognised that every element of fluid needs treatment. Although from the start pasteurised milk had to satisfy a plate count of less than 100,000 per ml, in the 1940s it became evident from emphasis on keeping quality that plate count standards had shortcomings. Since 1946 the official tests for pasteurisation efficacy became the phosphatase test and the modified methylene blue test. The phosphatase test still remains in use throughout the world but the methylene blue test has fallen out of use. During this period there were still some objections to pasteurisation, which are summarised in Table 12.1.

At that time the keeping quality of pasteurised milk was poor and its shelf-life was short. There was no widespread domestic refrigeration and milk was stored in the larder. At that time, a satisfactory keeping quality meant that milk should remain sweet and palatable for, say, 24 hours after delivery to the consumer and up to 48 h if you were lucky. If milk with a longer shelf-life was required, the only alternative was sterilised milk, with its strong cooked flavour and brown colour. Even in the 1960s, the choice of milk products was limited (*United*

Kingdom Dairy Facts and Figures (1964)). There was little mention of skim milk. In the UK, 69% of milk produced went to liquid sales, 31% to manufacture; 6.2% was raw, 18% went into condensed milk, less than 2.6% into other products, and yogurt received no mention. No breakdown was provided of what proportion of milk was pasteurised or sterilised and, at this juncture, the heat treatment regulations for UHT milk had only just been introduced.

HTST continuous processes were developed between 1920 and 1927 and for some time the ability of this process to produce safe milk was questioned. The importance of flow control and temperature control was known and it was appreciated that there was a distribution of residence times. Scales of operation were fairly substantial; Davis quotes HTST plants processing between 50 and 5000 gph (gallons per hour), although the most favoured were those of about 2000 gph (note that Imperial units were widely used: 1 gph = 4.54 litres per hour). Run times were cited as being up to 5 h. Cooling of milk to below 43ºF (5ºC) for distribution after pasteurisation was practised, and brine cooling was popular. Energy regeneration up to 72% was reported, and Davis (1955) reported that 75% of liquid milk was processed (pasteurised) using HTST methods. Gaskets were a problem on early equipment and, where milk was homogenised, it was run at a slightly higher temperature. Scale formation was also mentioned as being a problem. Time and temperature conditions that induced a cooked flavour and resulted in loss of cream line were well known. A report published slightly earlier by Cronshaw (1947) contains 200 pages of wisdom on milk pasteurisation with some interesting observations on vitamin losses and flavour changes during pasteurisation.

The role of pasteurisation in terms of inactivating *Mycobacterium tuberculosis* was well established. A key development was in 1927, when North and Park established a wide range of temperature/time conditions to inactivate tubercle bacillus (Cronshaw, 1947). These experiments were performed by heating milk heavily infected with tubercle bacilli in different conditions and injecting them into guinea pigs. A selection of conditions where negative results were found, i.e. those where the animals survived, were found at 212ºF (100ºC) for 10 s, at 160ºF (71.1ºC) for 20 s, at 140ºF (60ºC) for 10 min and at 130ºF (54.4ºC) for 60 min.

The phosphatase test had come into widespread general use as an index of correct heat treatment of milk, in particular to ensure that no milk was undertreated. It was developed from pioneering work reported by Kay and Graham in 1933 and was based upon the finding that the naturally occurring alkaline phosphatase in milk had similar inactivation kinetics to *Mycobacterium tuberculosis.*

Further developments were made in the classification of tests for evaluating the pasteurisation process, including tests for raw milk quality (platform test), pasteurisability (survival of thermodurics), efficiency of pasteurisation (pathogens and phosphatase), recontamination (thermophilic and coliform bacteria and the methylene blue test), and general bacterial quality, including organisms surviving pasteurisation plus contaminating organisms (plate count). The

methylene blue test is now little used, but the detection of alkaline phosphatase activity is still used as a statutory test in many countries.

It was also recognised that it would be more difficult to inactivate micro-organisms in situations where clumping occurred. The role of thermoduric and thermophilic microorganisms was recognised and it was fully appreciated that some microorganisms would survive pasteurisation. Maintaining the cream line was important as most milk was packaged into glass bottles. In fact, taking the temperature up to about 78°C was one method of losing the cream line. Odour and taste were also important quality characteristics. The role of post-pasteurisation contamination (ppc) was recognised, although this became more fully appreciated once pasteurised milk was stored refrigerated. Davis (1955) reported that when pasteurised milk sours or deteriorates rapidly that is almost invariably due to post-pasteurisation contamination.

A number of installations were introduced for pasteurising milk which had been sealed in bottles. Although the keeping quality was comparable (Davis, 1955), there were some major technical problems and costs were considered to be higher. Consequently, this innovation was relatively short-lived.

Davis (1955) wrote: 'In considering the history of pasteurisation, it is important to remember that, although scientists everywhere agreed fairly closely on the necessary degree of heat treatment, the process itself was very loosely controlled in commercial practice. Milk was frequently either overheated or underheated so that it either gave a cooked flavour or was found to contain viable tuberculosis bacteria. In addition, pasteurised milk was often so badly contaminated by unsterile plant, that its keeping quality was decreased.'

12.3   Major changes over the last fifty years

Some of the main changes that have influenced heat treatment of milk over the last 50 years are summarised below:

- A much wider variety of milk products are available, including skim, semi-skim, lactose-reduced, calcium-fortified and a whole range of speciality milk products with added health benefits.
- Milk from different species is much more widely available.
- Scales of operation have increased, with dairies handling upward of 5 million litres of milk a day, much of which would be heat-treated in some form or other.
- Considerable advances have been made in understanding the role of raw milk quality and the role of ppc in terms of improving keeping quality.
- Domestic refrigeration is much more widely available and refrigerated transport and storage systems have improved.
- With the onset of refrigeration there is a better understanding of the role of psychrotrophic bacteria, as raw milk remained refrigerated for longer periods prior to pasteurisation.

- Homogenisation is now widespread and there is a wider variety of packaging options.
- There is a demand for extended shelf-life products.
- Environmental issues have become more significant, in terms of reducing energy and water utilisation and reducing product waste; minimising effluent, reducing detergent usage and tackling one's carbon footprint are now considered to be important aims.

12.4   Pasteurisation equipment

12.4.1   Holder or batch heating

Cronshaw (1947) and Davis (1955) both provide excellent descriptions of equipment for the holder or batch process – individual vessels (heated internally) and externally heated systems with one or more holding tanks. These processes are labour-intensive and involve filling, heating, holding, cooling, emptying and cleaning. Temperatures attained are between 63.0 and 65.6ºC for 30 min. These processes are still used, particularly by small-scale producers. They are rela- tively time-consuming and labour-intensive: heating and cooling times are considerable, and the total time for one batch may be up to 2 hours. The factors influencing the heating and cooling times (t) arise from equating the rate of heat transfer from the heating medium to the rate at which the fluid absorbs energy. This is summarised in the following unsteady-state equation, which assumes perfect mixing and can be used to predict heating times, i.e. the time required to reach the pasteurisation temperature:

$$t = \frac{Mc}{AU} \ln \left(\frac{\theta_h - \theta_i}{\theta_h - \theta_f} \right)$$

where:

t = heating time (s)
c = specific heat (J kg^{-1} K^{-1})
M = mass of batch (kg)
A = surface area (m^2)
U = overall heat transfer coefficient (W m^{-1} K^{-1})
θ = temperature
i = initial; f = final; h = heating medium temperatures.

The dimensionless temperature ratio represents the ratio of the initial temperature driving force to that of the final approach temperature. The equation illustrates the exponential nature of the heat transfer process. The same dimensionless ratio can be used to evaluate cooling times, which tend to be longer, because of the limitations of chilled water temperature and, hence, the approach temperature. These can be shortened by using glycol systems, but this adds to the complexity. These factors have been discussed in more detail by Lewis (1990). One major advantage of the batch system is its flexibility, i.e. it is

easy to change from one product to another. Also, if the product is well mixed, there is no distribution of residence times.

In answer to the question 'Does HTST pasteurisation result in as good a bottle of milk as does the holder process?', Yale in 1933 concluded that one method of pasteurisation produces as good a bottle of pasteurised milk as does the other when sound methods are used and when conditions are comparable. I have not seen anything of late to contradict this, although most pasteurised milk is now produced by the HTST process. While pre-pasteurisation homogenisation is simple in a continuous flow system, it is more difficult to link with batch pasteurisation as the time delay between homogenisation and when the milk reaches pasteurisation temperature can result in an unacceptable amount of lipolysis. However, this problem can be largely overcome by homogenising the milk at $\geq 50^\circ$C (Deeth, 2002).

12.4.2   Continuous heating

HTST pasteurisation permits the use of continuous processing and regeneration of energy. The main types of indirect heat exchanger for milk are the plate heat exchanger and the tubular heat exchanger, with plate heat exchangers being by far the most common. Plate heat exchangers (PHE) are most widely used for pasteurisation of milk and cream and ice-cream mix. They have a high overall heat transfer coefficient (OHTC) and are generally more compact than tubular heat exchangers. Their main limitation is pressure, with an upper limit of about 20 bar. The normal gap width between the plates is between 2.5 and 5 mm but wider gaps are available for viscous liquids to prevent large pressure drops. In general, PHEs are the cheapest option and the one most widely used for low-viscosity fluids. Maintenance costs may be higher, as gaskets may need replacing, and the integrity of the plates also needs evaluating regularly, as pin-holes may appear in the plates of older heat exchangers. This may lead to pasteurised milk being recontaminated, for example if such plates are in the regeneration section, where a cracked or leaking plate may allow raw milk to contaminate already pasteurised milk. They are also more prone to fouling-related problems, but this is a more serious problem in UHT processing.

Tubular heat exchangers have a lower OHTC than plates and generally occupy a larger space. They have slower heating and cooling rates with a longer transit time through the heat exchanger. In general they have fewer seals and provide a smoother flow passage for the fluid. A variety of tube designs are available to suit different product characteristics. Most tubular plants use a multi-tube design. They can withstand higher pressures than PHEs. Although they are still susceptible to fouling, high pumping pressures can be used to overcome the flow restrictions. Tubular heat exchangers give longer processing times than PHEs with viscous materials and with products which are more susceptible to fouling. Thus they may be used with more viscous milk-based desserts. They are also widely used in UHT processing of milk and milk products.

Fig. 12.1   Heat exchange sections for HTST pasteuriser: 1, regeneration; 2, hot water section; 3, holding tube; 4, mains water cooling section; 5, chilled water cooling section.

The viscosity of the product is one major factor that affects the choice of the most appropriate heat exchanger and the selection of pumps. Viscosity will influence the pressure drop, causing a problem in the cooling section and when phase transition may take place, for example if coagulation or crystallisation takes place. For more viscous or particulate products, e.g. starch-based desserts or yogurts with fruit pieces, a scraped surface heat exchanger may be required. Viscosity data for a range of milk products at different temperatures are presented by Kessler (1981).

One of the main advantages of continuous systems over batch systems is that energy can be recovered in terms of regeneration. The layout for a typical regeneration section is shown in Fig. 12.1. The hot fluid can be used to heat the incoming fluid, thereby saving on heating and cooling costs. Regeneration efficiencies of over 90% can be obtained.

In terms of the temperatures depicted in Fig. 12.1, the regeneration efficiency (RE) is given by:

$$RE = \frac{\theta_2 - \theta_1}{\theta_3 - \theta_1} \times 100$$

where:

θ_1 = inlet temperature
θ_2 = temperature after regeneration
θ_3 = final temperature.

Although higher regeneration efficiency results in considerable savings in energy, it necessitates the use of higher surface areas, resulting from the lower-temperature driving force, and a slightly higher capital cost for the heat exchanger. This also means that the heating and cooling rates are also slower, and the transit times longer, which may affect product quality.

For milk containing substantial fat and for different cream products, homogenisation must be incorporated to prevent fat separation. Since homogenisation of raw milk is a very effective way of initiating lipolysis (Deeth and Fitz-Gerald, 2006), it must be carried out immediately before or after pasteurisation, which inactivates the native lipase. Homogenisation before pasteurisation is

(a)

(b)

*** Raw milk   ··· Pasteurised milk   ••• Hot water   —— Chilled water
▲▲▲ Steam   ——— Control pneumatic   – – – Control electrical

Fig. 12.2   (a) Layout of a typical HTST pasteuriser (with permission of Tetra Pak, Lund, Sweden); (b) schematic layout of HTST plant (reprinted with permission from M.A. Pearse, 'Pasteurization of liquid products', *Encyclopedia of Food Science and Nutrition*, p. 3445, copyright 1993, Academic Press).

preferable, as homogenisers can introduce post-pasteurisation contamination if used after pasteurisation.

The layout of a typical HTST pasteuriser and its accessory services is shown in Fig. 12.2. The holding time is controlled either by using a positive displacement pump or by a centrifugal pump linked to a flow controller, and the temperature is usually controlled and recorded. Note that a booster pump can be incorporated to ensure that the pasteurised milk is at a higher pressure than the raw milk in the regeneration section, to eliminate post-processing contamination in this section. A flow diversion valve diverts under-processed fluid back to the feed tank. In continuous processing operations there will be a distribution of residence times, and it is vital to ensure that the minimum residence time, i.e. the time for the fastest element of the fluid to pass through the holding tube, is greater than the stipulated time, to avoid under-processing. In a fully developed turbulent flow, the minimum residence time is about $0.83 \times$ average residence time (t_{av}). This will usually be the situation for milk, but it may be different for more viscous fluids. In this situation, the minimum residence time will only be $0.5 \times t_{av}$ and the distribution of residence times will be much wider.

Since most HTST pasteurisers are of the plate type, these should be regularly tested for leaks, as discussed earlier. Consideration should be given to ensuring that if leaks do occur, they do so in a safe fashion, i.e. pasteurised milk is not contaminated with cooling water or raw milk in the regeneration section. The control instrumentation, diversion valves and other valves should be checked regularly.

12.5   Determinants of keeping quality

The keeping quality of pasteurised milk is of prime importance both for the consumer and for the milk processor. Considerable advances have been made since those days when pasteurised milk would keep for only 24 hours. Tests for assessing whether pasteurised milk is still suitable for drinking include its sensory attributes (smell and taste), its microbial count (not higher than between 10^7 and 10^8 bacteria/ml), whether it forms a clot on boiling, and whether it is stable in 68% ethanol.

The following factors which influence keeping quality will now be discussed: raw milk quality, time–temperature processing conditions, reducing ppc and maintaining low temperatures during storage.

12.5.1   Raw milk quality

Raw milk is an extremely complex fluid, in terms of its chemical composition, its microbiological flora, its indigenous enzymes and its physical characteristics. Although water is the main component, it also contains proteins, fat, lactose, a wide range of vitamins and minerals and many other trace elements and minor components. In addition, there are numerous active enzymes including acid and alkaline phosphatases, lactoperoxidase and lactoferrin. The situation is more

complex because of biological variation. Milk from individual cows and from bulk milks will show day-to-day variations in composition, which are influenced by diet, temperature and the general well-being of the animal. Milk should also not be adulterated; one common adulterant is water, which is easily detected by measuring freezing point depression.

Milk is also available from other animals such as goats, sheep, buffaloes and camels, to name but a few, and will show wide differences in chemical composition.

Of particular interest is the heat stability of milk, and in situations where heat stability is poor, it may be necessary to resort to addition of citrate and phosphate salts to improve it. This is usually more relevant to UHT treatment but it is also applicable to calcium fortification of milk, which can easily lead to poor heat stability, especially if the calcium salts selected reduce milk pH and increase ionic calcium. This is currently one of the author's main research interests.

12.5.2 Microbiological aspects

Raw milk from healthy animals has a very low microbial count, but it easily becomes contaminated with spoilage and perhaps some pathogenic microorganisms. These need to be inactivated and this is readily achieved by heat treatment. From a milk processor's standpoint, raw milk composition and its microbial loading will vary from day to day.

Raw milk may contain pathogenic microorganisms picked up from the farm environment, including vegetative bacteria such as *Staphylococcus aureus*, *Campylobacter jejuni*, *Salmonella* spp, *Escherichia coli* and *Yersinia enterocolitica*, and spore formers such as *Bacillus cereus* and *Clostridium* spp. It is considered that these vegetative pathogens can be effectively controlled by pasteurisation and that they are not a major determinant of keeping quality. According to Codex Alimentarius (2003), pasteurisation will achieve 5 decimal reductions of *C. burnetii*, so inactivation of *Campylobacter* and *Salmonella*, both of which have been reported to cause food poisoning outbreaks in milk, will be higher. *Listeria* spp. are also inactivated. Pasteurisation also reduces the population of acid-producing spoilage bacteria and coliform bacteria, including *E. coli* O157. A summary of heat resistance data for different pathogens is provided by Lewis and Heppell (2000).

Of further interest is what survives pasteurisation. Thermoduric bacteria are defined as those which survive 63ºC for 30 min, whereas spore-forming bacteria can survive 80ºC for 10 min. *Bacillus cereus* spores are relevant here, being the main spore-forming pathogen which will survive pasteurisation and grow at low temperature. It will certainly cause spoilage in heat-treated milk, and may cause bitty cream and produce an intense bitter flavour, but it rarely causes food poisoning because contaminated products are so unacceptable.

Spore counts in raw milk have been rarely reported to exceed 10^3/ml, although Bramley and McKinnon (1990) reported that they may reach 5000/ml.

They are higher in winter than in summer. In a more recent survey on spore-forming bacteria, mesophilic spore formers were found to be the most predominant, with a mean value of 7600 spores/ml but occasional counts of over 2.4×10^5/ml. Psychrotrophic spore counts were very low, with a maximum of 3.5 spores/ml, and thermophilic spores slightly higher with a maximum of 54 spores/ml (McGuiggan *et al.*, 2002). Suggested reasons for the higher results were the improved recovery techniques used.

12.5.3   Enzyme inactivation

Milk contains an abundance of enzymes and their inactivation in the pasteurisation region has been the subject of two major reviews by Griffiths (1986) and Andrews *et al.* (1987).

The role of alkaline phosphatase has been discussed earlier in this chapter. The original phosphatase test for assessing the adequacy of pasteurisation was based upon the reaction of phosphatase with disodium phenyl phosphate. It was claimed to be able to detect the presence of about 0.2% raw milk (addition) in pasteurised milk, as well as under-processing, for example 62°C instead of 62.8°C for 30 min or 70°C rather than 72°C for 15 s. Since then, a more auto-mated test based on fluorescence measurement (e.g. Fluorophos) has increased the sensitivity of the method further, being able to detect the presence of 0.006% added raw milk. This is a very useful quality assurance test procedure and its introduction should further help detect low levels of post-pasteurisation con-tamination, which should also reduce the incidence of pathogens in pasteurised milk.

Griffiths determined the heat resistance of several indigenous milk enzymes. Alkaline phosphatase was the most heat labile of those measured ($D_{69.8} = 15$ s; $z = 5.1$°C), compared to lactoperoxidase ($D_{70} = 940$ s; $z = 5.4$°C). Acid phosphatase was much more heat resistant than alkaline phosphatase (about 100-fold). Some discrepancies were also noticed between data obtained from capillary tube experiments and that obtained from HTST conditions using plate heat exchangers. Lactoperoxidase activity was thought to provide a useful indicator of over-processing. Activities determined on a plate heat exchanger (PHE) for 15 s were generally lower than those obtained from the laboratory data. Using a PHE, lactoperoxidase activity was almost destroyed at 78°C for 15 s and completely destroyed at 80°C for 5 s. The enzyme appeared sensitive to temperatures above 75°C, with a *z*-value of 5.4°C. Since that work, ensuring milk has a positive lactoperoxidase activity has become part of the milk pasteurisation regulations of several countries.

Ribonuclease was found to be more heat resistant than lactoperoxidase. Again there were discrepancies between laboratory studies and PHE studies. No loss of activity was observed at 80°C for 15 s (lab), whereas a 40% loss of activity was found in a PHE at 80°C for 15 s. Andrews *et al.* (1987) determined the following retention of activities for milk samples heated for precisely 15 s at 72°C in glass capillary tubes: acid phosphatase, >95%; α-D-mannosidase,

98%; xanthine oxidase, 78%; γ-glutamyl transpeptidase, 75%; α-L-fucosidase, 26%; N-acetyl glucosamidase, 19%; and lipoprotein lipase, 1%. It was recommended that N-acetyl-β-glucosamidase could be used for more detailed studies between 65 and 75ºC and γ-glutamyl transpeptidase (GGTP) between 70 and 80ºC.

Patel and Wilbey (1994) recommend measuring GGTP activity for assessing the severity of HTST heat treatments above the minimum for whole milk, skim milk, sweetened milks, creams and ice-cream mixes. There was a good correlation between the reduction in GGTP activity, destruction of streptococci and water activity.

Indigenous lipases may give rise to soapy off-flavours, especially if raw milk is subjected to excessive agitation at temperatures of about 50ºC, e.g. when mixing flavoured milks or other similar products. An academic visitor from the US once pointed out that he could detect a low level of lipolysis in pasteurised milk in the UK, presumably caused by agitation during transportation and processing. However, this does not bring complaints from UK consumers and so raises the issue of different levels of sensitivity arising from exposure to the same product processed in different countries.

Plasmin and plasminogen are indigenous proteases in milk. Plasmin is very heat resistant and will survive pasteurisation, but plasmin-related problems have not been commonly reported in pasteurised milk, again probably because of its short shelf-life and low storage temperature. Plasmin activity may also be controlled by naturally occurring plasmin inhibitors, although it has been claimed that these are inactivated at temperatures between 75 and 85ºC. Problems related to residual plasmin activity are more serious in UHT milk.

Enzymes may also arise from psychrotrophic bacteria. These enzymes are also likely to survive pasteurisation, as they are very heat resistant. However, it is unlikely that residual bacterial lipases and proteases will cause problems in pasteurised milks because of their relatively short shelf-life and refrigerated storage conditions. In general, however, it is best to avoid older milk for pasteurisation for many reasons, including higher microbial count, higher acidity (lower pH) and reduced heat stability, higher enzyme activity and more likelihood of off-flavours.

12.5.4   Processing conditions

Normal HTST conditions for milk are 71.7ºC for 15 s. One interesting question relates to the use of more severe processing conditions for pasteurisation. Perhaps unexpectedly, using higher temperatures for pasteurisation has been shown to reduce its keeping quality. This has been identified by Kessler and Horak (1984), Schroder and Bland (1984) and Schmidt *et al.* (1989), and it is one that should be revisited, since it would be logical to expect a more severe heating process to result in an improved keeping quality. More recently Gomez Barroso (1997) and Barrett *et al.* (1999) showed that milks heated at 80ºC for 15 s in general had a reduced keeping quality compared to milk heated at 72ºC

for 15 s. One explanation for this surprising observation is that the more severe conditions cause heat shocking of the spores and that their activity then reduces the keeping quality. However, experimental evidence for this is not so clear. An alternative explanation is that the lactoperoxidase system (LPS) also plays a role. The LPS system involves the enzyme lactoperoxidase (LP), hydrogen peroxide and thiocyanate, all of which are present in raw milk. The oxidation products, e.g. hypothiocyanite, exhibit strong antimicrobial activity by oxidising sulphydryl groups of bacterial cell walls (Reiter and Harnulv, 1982). The LPS system can be further activated in raw milk by small additions of thiocyanate and hydrogen peroxide and can be used to keep raw milk longer in countries where refrigeration is not widespread (*IDF Bulletin*, 1988). Lactoperoxidase inactivation is very temperature sensitive with *z*-values of about 4ºC. Some heat treatment regulations now require that pasteurised milk should show a positive lactoperoxidase activity. Marks *et al.* (2001) showed that pasteurisation conditions of 72ºC for 15 s, resulting in an active lactoperoxidase system, were found to greatly increase the keeping quality of milks inoculated with *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus thermophilus*, when compared to heating at 80ºC for 15 s. However, pasteurisation temperature had no effect on the keeping quality of milks challenged with *Bacillus cereus* spores. Double pasteurisation processes have not been found to be effective (Brown *et al.*, 1979) and therefore are rarely used. It may be possible to exploit some other natural anti-microbial systems in raw milk. These have been described in more detail by the IDF (1994).

12.5.5   Process characterisation

A number of parameters have been used to characterise pasteurisation processes. One parameter is the pasteurisation unit (PU). One pasteurisation unit results from a temperature of 60ºC (140ºF) for 1 min. The equivalent *z*-value is 10ºC (18ºF), which is high for vegetative bacteria. Thus, the number of pasteurisation units for a heating temperature T (ºC) and heating time t (min) is given by:

$$PU = 10^{(T-60)/10} \cdot t$$

Thus, a temperature of 63ºC for 30 min would have a value of approximately 60 PU (Wilbey, 1993), whereas HTST conditions (72ºC for 15 s) would give only 3.96 PU. This discrepancy arises from the large *z*-value: perhaps the lesson to be learnt is that it may not be meaningful to extrapolate this to continuous pasteurisation processes.

Another parameter, introduced by Kessler, is p^*. This is based on a reference temperature of 72ºC and a *z*-value of 8ºC (K). Processing conditions of 72ºC for 15 s are designated as providing a safe pasteurisation process for milk and are given an arbitrary p^* value of 1. It can be calculated from:

$$p^* = \frac{10^{(T-72)/z}}{15} t$$

where T = temperature (°C); t = time (s). Figure 12.3 shows the time–temperature combinations that correspond to a $p*$ value of 1 (normal pasteurisation) as well as other $p*$ values (0.1 to 10).

This simplified equation ignores the contribution of the heating and cooling section. Both these factors provide an additional measure of safety and are further discussed by Kessler (*IDF Bulletin*, 1986). Knowledge of the heating and cooling profiles will enable their contribution to be determined. The procedure for this is from the temperature–time profile to plot p against time and determine

Fig. 12.3   Temperature–time conditions giving rise to different $p*$ values (reprinted with permission from H.G. Kessler, 'Effect of thermal processing on milk', *Developments in Food Preservation*, vol. 5, S. Thorne (ed.), pp. 91–130, copyright 1989, Verlag A. Kessler).

the area under the curve. Alternatively, the activation energy (285 kJ/mol) can be used. As mentioned earlier, it is important to check the minimum residence time; dye injection methods can be used to check this. It is also important to calibrate temperature probes at regular intervals.

It is pertinent to comment on the fact that these different pasteurisation parameters make use of different z-values: direct comparison of holder (63ºC/30 min) and HTST conditions (72ºC/15 s) would give a z-value of about 4.3ºC. It is probably not to be recommended to extrapolate PU from a batch to a continuous process, or p^* from continuous to batch processes. The z-values used are:

Comparing holder/HTST process	4.3
p^*	8
PU	10

However, pasteurisation conditions used in some situations often exceed 71.7ºC for 15 s. From the above discussion, it is not clear to the author what the rationale is for selecting higher processing temperatures. It may be that they are being used to overcome problems related to poor quality milk, or poor control of post-pasteurisation contamination. This is worthy of further debate.

It should also be mentioned that pasteurisation conditions do vary from one country to another. In the USA, a wide range of conditions are used, including 63ºC for 30 min, 77ºC for 15 s, 90ºC for 0.5 s and 100ºC for 0.01 s.

12.5.6   Post-pasteurisation contamination (ppc)

This is now considered to be a very important determinant of keeping quality (KQ), and Muir (1996a,b) describes how this was recognised both for milk and for cream in the early 1980s. Post-pasteurisation contamination encompasses the recontamination of the product anywhere downstream of the end of the holding tube. It can occur in the regeneration or cooling sections, in storage tanks and in the final packaging of the product, due to poor hygienic practices. It can greatly be reduced by ensuring that all internal plant surfaces in contact with the product are heated at 95ºC for 30 min. It can only be completely eliminated by employing aseptic techniques downstream of the holding tube. One of the main safety concerns is recontamination of the product with pathogens from raw milk, and this could occur due to bypassing of the holding tube by a number of possible routes, including pinhole leaks in plates. In terms of reducing keeping quality, preventing recontamination with Gram-negative psychrotrophic bacteria is likely to be very important. The presence in pasteurised products of high counts of microorganisms (e.g. coliform bacteria), which should be inactivated by pasteurisation, is indicative of ppc. An International Dairy Federation publication (*IDF Bulletin*, 1993) has catalogued a large number of tests which can be used to determine the extent of the problem. In practical situations where the KQ of milk starts to deteriorate or is below expectations, the most likely explanation would be an increase in ppc and this should be the first factor to be investigated.

12.5.7   Storage temperature

In general the lower the storage temperature, the better will be the keeping quality, bearing in mind the costs and practical problems of ensuring low temperatures throughout the cold chain and in domestic refrigerators.

Later experiments confirmed that pasteurised milk produced from high quality raw milk could be stored for up to 20 days at 8ºC, for between 30 and 40 days at 4ºC and for upwards of 60 days at 2ºC. However, it must be emphasised that these experiments were performed with good quality raw milk, i.e. the counts immediately after pasteurisation were never above 10^3/ml, even for raw milk which had been stored for 8 days at 4ºC prior to pasteurisation. These results also illustrate that good keeping quality can be achieved by eliminating ppc and can be further enhanced by using low storage temperatures.

Before domestic refrigeration was commonplace, Cronshaw (1947) reported that pasteurised milk would keep for about 24 h. Household refrigeration helped to improve this considerably and in the UK by 1957, 10% of households had a refrigerator, increasing to 30% by 1962 and up to 90% by 1979. Raw milk is stored at typically 4ºC; temperatures in the cold chain are slightly higher and they are likely to be higher still in domestic refrigerators.

12.6   Other changes during pasteurisation

There are some other important changes taking place during pasteurisation. In general, as far as chemical reactions are concerned, pasteurisation is a mild process: about 5–15% of the whey protein is denatured in milk; this is not sufficient to significantly increase the levels of free sulphydryl group activity or to induce formation of hydrogen sulphide and lead to the development of any cooked flavour. Whey protein denaturation is higher in skim milk concentrates produced by ultrafiltration, increasing with the increase in the concentration factor (Guney, 1989). There is some suggestion that the holder process may be slightly more severe than the HTST process in these respects (Painter and Bradley, 1996). Pasteurisation results in little change in renneting properties and little association of whey proteins with casein, no dephosphorylation and no significant reduction in ionic calcium. Thus, it is possible to make good Cheddar cheese from pasteurised milk and the majority of milk for cheesemaking in the UK is subject to pasteurisation. However, significant amounts of cheese are still made from raw milk in some countries.

The effects on heat-sensitive vitamins and other components are also very small. Overall, pasteurisation results in little change in texture, flavour and colour, compared to raw milk. Wilson, as far back as 1942, reported that it was clear that the majority of people are unable to distinguish between raw and pasteurised milk. Also, the difference in taste between different raw milks appears to be as great as or greater than the difference between raw and pasteurised milks. There is no evidence to suggest that this observation has changed over the past 65 years. Nursten (1995) reports that pasteurisation barely

alters the flavour of milk and that the volatile flavours responsible for cooked flavour were negligible.

12.7   Further issues during pasteurisation

Pasteurisation was introduced to inactivate *Mycobacterium tuberculosis* and to control *Coxiella burnetti*. Tuberculosis in cattle and badgers is again becoming an issue, due to *Mycobacterium bovis*, as is the presence of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) and whether it would survive pasteurisation. MAP levels found in raw milk appear to be low, but there is no real indication of true levels because of the decontamination procedures used to remove the other bacteria in raw milk and its extremely slow growth rate. MAP levels found in milks subjected to pasteurisation are also low but there are many inconsistencies in the experimental results (Grant *et al.*, 2001; Hammer *et al.*, 1998).

Using the holder process (63°C for 30 min), most investigators found some survivors after pasteurisation, but inoculum levels were much higher than would be found in raw milk. The D_{63} values quoted were 2.7–2.9 min, which would give a high level of inactivation (12.4 log reductions) during pasteurisation, and would provide a more than adequate process. Most other results suggest that the holder process was not so efficient as this. Tails were also found in the survivor curves, which implied the presence of a more heat-resistant sub-population, though this could be an artifact. Results from HTST studies are also inconsistent and suggest great variability in the heat resistance data. One report suggested a D_{72} value of 11.7 s. According to this, normal HTST conditions would only achieve about 1.3 log reductions, which would mean that all samples inoculated with 100 cfu/ml MAP would show surviving MAP after pasteurisation. However, results from milks inoculated with 10^7 and 10^4 cfu/ml indicated that about 20% and 40% of samples contained no viable MAP after HTST treatment, which suggested at least 7 and 4 decimal reductions (respectively) in some of these samples. This is inconsistent with a D_{72} value of 11.7 s.

Experiments also suggested that MAP inactivation is not temperature sensitive, although conclusions were based on the percentage of surviving bacteria rather than the numbers of decimal reductions achieved. There was a 55% survival at 72°C for 15 s and experiments at 75, 78, 80, 85 and 90°C also showed measurable survivor rates. The survival rates appeared to be higher after heat treatments at 80°C than at 75°C and 78°C. At first sight this is unexpected but it could demonstrate that MAP is inhibited by an active lactoperoxidase system, which would be inactivated at 80°C. This apparent lack of temperature dependence is unusual in a bacterium and is worthy of further investigation, as is any protective effect that may be conferred by the lactoperoxidase system (Marks *et al.*, 2001).

Results from surveys on raw milks and pasteurised milks are also inconclusive in that MAP was found in 2% of both raw and pasteurised milk samples tested. This again would suggest that pasteurisation is having no significant

effect. Clearly, the heat-resistance data generated to date for MAP are inconclusive and do not permit an accurate assessment of the efficacy of the pasteurisation process with regard to MAP. Information has been published by the *IDF Bulletin* (1998). In the UK it has been recommended that HTST pasteurisation conditions should be increased to 72ºC for 25 s as part of a strategy for controlling MAP in cows' milk (Food Standards Agency, 2002). Hickey (2009) has recently pointed out that while this recommendation has been widely adopted by the UK industry, and supported by many retailers, it is a recommendation that is voluntary and is not a legal requirement for HTST heat treatment, which remains at 72ºC for 15 s. The whole situation regarding MAP has been reviewed by Griffiths (2006).

12.8   Pasteurisation of some other milk-based products

As well as providing safe market milk, pasteurisation is crucial to many processes, for example cheesemaking, ice-cream manufacture and powdered milk production, to ensure that these are free of pathogenic microorganisms. Other pasteurised products include creams and ice-cream mix and, in the UK, the minimum temperature–time conditions for these products are 72ºC for 15 s and 79ºC for 15 s, respectively, although conditions for cream products are more severe in some other countries. One major difference between milk and creams or ice-cream mix is the higher viscosity of the latter products, which will result in lower Reynolds numbers and perhaps even streamline rather than turbulent flow. If there is a transition from turbulent to streamline flow, this will alter the residence time distribution and significantly reduce the minimum residence time achieved.

12.9   Legislation and control

The practicalities of enforcing the legislation for heat-treated market milk vary from country to country. Control of pasteurisation processes is mediated through heat treatment regulations, which are based upon the long-known requirement that pasteurised milk should be produced under conditions which exceed a certain temperature for certain times. Pasteurised milk should also show negative phosphatase activity and there may also be a need for it to be lactoperoxidase positive, otherwise it might be labelled high pasteurised.

Regulations may also include details of microbial counts. More general requirements are that the milk should not be watered down or contaminated. Thus, freezing point depression remains important for raw milk quality. Another adulterant which has attracted recent attention in China is melamine. This was added to milk to boost its apparent protein, presumably because it had been diluted with water. The scale of this adulteration has obviously been massive. The fact that the milk had been diluted would have been detected by freezing

point depression. Had this been applied widely, it might have alerted the authorities that such practices were occurring at a much earlier stage. Although not directly related to pasteurisation, it does draw attention to ensuring that raw milk to be used for processing is not adulterated in any form.

There has also been a move towards employing HACCP principles to control and reduce pasteurisation failures. Miller Jones (1992) documents a major pasteurisation failure in the USA, where over 16,000 people were infected with salmonella and 10 killed. The cause was believed to be a section of the plant which was not easy to drain and clean and lead to pasteurised milk being recontaminated.

For the latest thinking in terms of such control measures, the reader is referred to New Zealand draft regulations (http;//www.nzfsa). This includes corrections to pasteurisation times to account for particles in the range from less than 200 microns up to 1000 microns. They also suggest that for turbulent flow, the maximum velocity may be assumed to be 1.33 times the average velocity for a Reynolds number of 4000 and 1.25 times the average velocity when the Reynolds number exceeds 20,000. These correspond to t_{min}/t_{av} ratios of 0.75 and 0.80, respectively. Competence requirements for heat treatment evaluators and risk management programme verifiers are also covered. Further discussion on heat treatment legislation is provided by Komorowski (2006) and most recently by Hickey (2009).

12.10   Extended shelf-life milk

Many consumers, including the majority in the UK, do not like the flavour (referred to as cooked, boiled or cabbagey) that is associated with milk that has been too severely heat treated, such as UHT and sterilised milk, so it is important to minimise this cooked flavour intensity. There are some early references to time–temperature conditions required to induce a cooked flavour (Cronshaw, 1947). Where there is a requirement to further increase the shelf-life of heat-treated products, either for the convenience of the processor and the consumer or to provide additional protection against temperature abuse, more severe heating conditions can be used. However, it is important to avoid the development of a cooked flavour, which would result from higher temperatures. It is the author's experience that this occurs at a temperature of about 85–90ºC for 15 s. Therefore one approach is to use temperatures above 100ºC for shorter times. Wirjantoro and Lewis (1996) showed that milk heated to 115ºC for 2 s had a much better keeping quality than milks heated at both 72ºC for 15 s and 90ºC for 15 s. There is no doubt that temperatures in the range 115–120ºC for 1–5 s are more effective than temperatures below 100ºC for extending the shelf-life of refrigerated products.

A second approach is to use small amounts of a bacteriocin, such as nisin. The addition of small amounts of nisin (40 IU/ml) has been found to be effective in reducing microbial growth following heat treatment at 72ºC for 15 s and more

so at 90ºC for 15 s. It was particularly effective at inhibiting *Lactobacillus* at both temperatures. Results for milk heat treated at 117ºC for 2 s with 150 IU/ml nisin were even more spectacular. Such milks have been successfully stored for over 150 days at 30ºC with only very low levels of spoilage (Wirjantoro *et al.*, 2001). Local regulations would need to be checked to establish whether nisin is a permitted additive in milk and milk-based beverages.

As a word of caution, it is important to eliminate post-pasteurisation contamination, as nisin is not effective against Gram-negative contaminants, such as pseudomonads (Phillips *et al.*, 1983; Wirjantoro *et al.*, 2001).

Direct processes, either injection or infusion processes, offer an alternative solution to this problem and milks processed by this method at 138ºC for 2–4 s are known as ultrapasteurised in the USA. However, such products, although having low cooked flavour intensity, will be subjected to much higher whey protein denaturation compared to conventional pasteurisation and their functionality may be impaired. A further strategy is to store pasteurised products at 2ºC, rather than 5–7ºC. This would further increase keeping quality but it may not be practicable to do this.

12.11   Conclusions

HTST pasteurisation is an effective method of making milk safe for consumption, without unduly changing either its sensory characteristics or its nutritional value. As a continuous process, it makes use of energy regeneration and it is capable of both scales of operation and energy efficiencies that cannot be matched by alternative processes. The most important determinants to ensure good keeping quality are raw material quality, ensuring time–temperature conditions, reducing or eliminating post-pasteurisation contamination, and ensuring that a low temperature is maintained during storage. Most HTST pasteurisers are of the plate type and these should be tested periodically for leaks; if leaks do occur, they should do so in a safe fashion, i.e. so that pasteurised milk is not contaminated with cooling water or raw milk in the regeneration section. The control instrumentation, diversion valves and other valves should be checked regularly. Pasteurisation is crucial to many processes, for example cheese-making, ice-cream manufacture and powdered milk production, to ensure that these are free of pathogenic microorganisms. Again, in all these processes, it is crucial to reduce ppc, to ensure the best quality products.

12.12   References

ANDREWS A T, ANDERSON M and GOODENOUGH P W (1987), A study of the heat stabilities of a number of indigenous milk enzymes, *Journal of Dairy Research*, 54, 237–246.
BARBOSA-CANOVAS G V, POTHAKAMURY U R, PALOU E and SWANSON B G (EDS) (1998), *Nonthermal Preservation of Foods*, Marcel Dekker, New York.

BARRETT N, GRANDISON A S and LEWIS M J (1999), Contribution of lactoperoxidase to the keeping quality of pasteurized milk, *Journal of Dairy Research*, 66, 73–80.

BRAMLEY A J and MCKINNON C H (1990), The microbiology of raw milk, in *Dairy Microbiology, Volume 1, The Microbiology of Milk*, Robinson R K (ed.), Elsevier Applied Science, London, pp. 163–208.

BROWN J V, WILES R and PRENTICE G A (1979), The effect of a modified Tyndallization process upon the sporeforming bacteria of milk and cream, *Journal of Society of Dairy Technology*, 32, 109–112.

CODEX ALIMENTARIUS (2003), Proposed Draft Code of Hygienic Practice for Milk and Milk Products at Step 5 (ALINORM 03/13A, Appendix III).

CRONSHAW H B (1947), *Dairy Information*, Dairy Industries Ltd, London.

Daily Telegraph (2008), Saturday 3 May.

DAVIS J G (1955), *A Dictionary of Dairying*, 2nd edition, Leonard Hill, London.

DEETH H C (2002), Lipolysis in milk and dairy products – A research journey, *Food Australia*, 54, 433–436.

DEETH H C and FITZ-GERALD C H (2006), Lipolytic enzymes and hydrolytic rancidity, in *Advanced Dairy Chemistry, Volume 2, Lipids*, Fox P F and McSweeney P (eds), Springer, New York, pp. 481–556.

FOOD STANDARDS AGENCY (2002), Consultations, *Draft strategy for the control of Mycobacterium avium subsp. paratuberculosis (MAP) in cows' milk*.

GOMEZ BARROSO B (1997), *Effect of raw milk quality on keeping quality of pasteurized milk*, MSc dissertation, Department of Food Science and Technology, University of Reading, UK.

GRANT I R, ROWE M T, DUNDEE L and HITCHINGS E (2001), *Mycobacterium avium* ssp. *paratuberculosis*: its incidence, heat resistance and detection in raw milk and diary products, *International Journal of Dairy Technology*, 54(1), 2–13.

GRIFFITHS M W (1986), Use of milk enzymes and indices of heat treatments, *Journal of Food Protection*, 49, 696–705.

GRIFFITHS M W (2006), *Mycobacterium paratuberculosis*, in *Emerging Foodborne Pathogens*, Motarjemi Y and Adams M (eds), Woodhead Publishing, Cambridge, pp. 522–556.

GUNEY P (1989), PhD thesis, University of Giessen.

HAMMER P, KNAPPSTEIN K and HAHN G (1998), Significance of *Mycobacterium paratuberculosis* in milk, *IDF Bulletin* No. 330, International Dairy Federation, Brussels, pp. 12–16.

HICKEY M (2009), Current legislation of market milks, in *Milk Processing and Quality Management*, Tamine A Y (ed.), SDT, Wiley-Blackwell, Chichester, pp. 101–138.

IDF (1994), *Indigenous Antimicrobial Agents of Milk – Recent Developments*, IDF Reference SI 9404.

IDF Bulletin (1986), Monograph on pasteurised milk, No. 200.

IDF Bulletin (1988), Code of practice for the preservation of milk by the lactoperoxidase system, No. 234.

IDF Bulletin (1993), Catalogue of tests for the detection of ppc of milk, No. 281.

IDF Bulletin (1998), Significance of *Mycobacterium paratuberculosis* in milk, No. 330.

KESSLER H G (1981), *Food Engineering and Dairy Technology*, Verlag A Kessler, Freising, Germany.

KESSLER H G and HORAK F P (1984), Effect of heat treatment and storage conditions on keeping quality of pasteurized milk, *Milchwissenschaft*, 39, 451–454.

KOMOROWSKI E S (2006), New dairy hygiene legislation, *International Journal of Dairy*

Technology, 59, 97–101.

LEWIS M J (1990), *Physical Properties of Foods and Food Processing Systems*, Woodhead Publishing, Cambridge.

LEWIS M J and HEPPELL N (2000), *Continuous Thermal Processing of Foods: Pasteurization and UHT Sterilization*, Aspen Publishers, Gaithersburg, MD.

MARKS N E, GRANDISON A S and LEWIS M J (2001), Challenge testing of the lactoperoxidase system in pasteurized milk, *Journal of Applied Microbiology*, 91, 735–741.

MCGUIGGAN J T M, MCCLEEREY D R, HANNAN A and GILMOUR A (2002), Aerobic spore-forming bacteria in bulk raw milk: factors influencing the numbers of psychrotrophic, mesophilic and thermophilic *Bacillus* spores, *International Journal of Dairy Research*, 55, 100–107.

MILLER JONES J (1992), *Food Safety*, Egan Press, St Paul, MN.

MUIR D D (1996a), The shelf-life of dairy products: 1 Factors influencing raw milk and fresh products, *Journal of the Society of Dairy Technology*, 49(1), 24–32.

MUIR D D (1996b), The shelf-life of dairy products: 2 Raw milk and fresh products, *Journal of the Society of Dairy Technology*, 49(2), 44–48.

New Zealand draft regulations, http://www.nzfsa.govt.nz/dairy/publications/consultation/heat-treatment-draft-22-aug

NURSTEN H (1995), Heat-induced changes in the flavour of milk, in *Heat-induced Changes in Milk*, 2nd edition, Fox P F (ed.), IDF Reference 9501, International Dairy Federation, Brussels.

PAINTER C J and BRADLEY JR, R L (1996), Residual alkaline phosphatase activity in milks subjected to various time/temperature treatments, in *Heat Treatments and Alternative Methods*, IDF, IDF/FIL No. 9602, pp. 396–402.

PATEL S S and WILBEY R A (1994), Thermal inactivation of γ-glutamyltranspeptidase and *Enterococcus faecium* in milk-based systems, *Journal of Dairy Research*, 61, 263–270.

PHILLIPS J D, GRIFFITHS M W and MUIR D D (1983), Effect of nisin on the shelf-life of pasteurized double cream, *Journal of the Society of Dairy Technology*, 36, 17–21.

REITER B and HARNULV B G (1982), The preservation of refrigerated and uncooled milk by its natural lactoperoxidase system, *Dairy Industries International*, 47, 13–19.

SATIN M (1996), *Food Irradiation – A Guidebook*, Technomic Publishing Co., Lancaster, PA.

SCHMIDT D, CROMIE S J and DOMMETT T W (1989), Effects of pasteurisation and storage conditions on the shelf-life and sensory quality of aseptically packaged milk, *Australian Journal of Dairy Technology*, 44(1), 19–24.

SCHRODER M A and BLAND M A (1984), Effect of pasteurisation temperature on keeping quality of whole milk, *Journal of Dairy Research*, 51, 569–578.

SUN D-W (ed.) (2006), *Thermal Food Processing, New Technology and Quality Issues*, CRC, Taylor & Francis, Boca Raton, FL.

United Kingdom Dairy Facts and Figures (1964), The Federation of United Kingdom Milk Marketing Boards.

VILLAMIEL M, SCHUTSYER M A I and DE JONG P (2009), Novel methods of milk processing, in *Milk Processing and Quality Management*, Tamine A Y (ed.), SDT, Wiley-Blackwell, Chichester, pp. 205–236.

WILBEY R A (1993), Pasteurisation of foods: (a) Principles of pasteurisation, in *Encyclopedia of Food Science, Food Technology and Nutrition*, Academic Press, London, pp. 3437–3441.

WILSON G S (1942), *The Pasteurisation of Milk*, Edward Arnold, London, p. 104.

WIRJANTORO T I and LEWIS M J (1996), Effect of nisin and high temperature pasteurisation on the shelf-life of whole milk, *Journal of the Society of Dairy Technology*, 4, 99–102.

WIRJANTORO, T I, LEWIS M J, GRANDISON A S, WILLIAMS G C and DELVES-BROUGHTON J (2001), The effect of nisin on the keeping quality of reduced heat treated (RHT) milks, *Journal of Food Protection*, 64, 213–219.

13

Improving UHT processing and UHT milk products

H. Deeth, University of Queensland, Australia

Abstract: Ultra-high-temperature (UHT) processing of milk converts a very perishable natural product into a long-shelf-life product. This chapter first discusses the principles, the various processes used and some recent trends in the technology. It highlights the significance of the temperature–time profile of the UHT process and relates this to changes that occur during processing, including whey protein denaturation and formation of protein complexes, fouling or deposit formation, Maillard reactions and lactose isomerisation. Finally, the changes that occur during storage such as gelation, protein changes, flavour changes and fat separation are discussed.

Key words: ultra-high-temperature (UHT) processing, temperature–time profile, whey protein denaturation, fouling, flavour, gelation.

13.1 Introduction

The ultimate goal of ultra-high-temperature (UHT) processing of milk is to convert a very perishable natural product into a product which will remain virtually unchanged without refrigeration for an extended period of time, e.g. 6–12 months. In addition, the treated product should have sensory and nutritional characteristics not unlike the raw product or a product subjected to a mild heat treatment. These lofty goals have, to date, eluded the dairy processing industry but developments over the years have greatly improved both UHT processing efficiency and product quality since the technology was introduced commercially in 1953 (Hostettler, 1972).

Two major factors influenced the introduction of UHT processing of milk. The first was the desire to produce a shelf-stable, safe product with better sensory characteristics than in-container sterilised milk; this milk has a very distinct heated flavour and a brownish colour. The second factor was the development of aseptic packaging. High-temperature heat processing had been available for some time but it was not until aseptic packaging was commercialised that the treated milk could be effectively stored at room temperature without spoilage (Robertson, 2003). The importance of the introduction of aseptic packaging was recognised in 1989 by the Fellows of the Institute of Food Technologists as the 'most significant food science innovation of the last 50 years' (Robertson, 2002).

UHT processing is now a mature technology but its use throughout the world is variable. In some European countries, such as Portugal, Spain and France, UHT milk represents over 80% of milk marketed for direct consumption, while in other countries such as the USA, the UK, Australia and New Zealand, it constitutes less than 10% of the liquid market milk. The reasons for the difference are varied but include historical, cultural and pricing factors. In addition, in countries with a relatively low consumption of UHT milk, a large percentage of consumers has a strong preference for the flavour of pasteurised milk over UHT milk (Perkins and Deeth, 2001).

UHT processing with associated aseptic packaging is a very demanding technology and presents many scientific and technical challenges. This chapter discusses many of these and outlines developments which have been introduced to address them.

13.1.1   Trends in UHT processing

Since the introduction of UHT processing combined with continuous aseptic packaging, the technology has been reasonably stable and many of the challenges facing UHT processors have remained. These include minimising heat-induced flavours, destroying bacterial spores and enzymes, particularly proteinases, while avoiding unacceptable chemical change, and minimising fouling or burn-on on heat exchangers. However, new challenges have also arisen. A major one was the identification in UHT milk of *Bacillus sporothermodurans*, a mesophilic sporeformer, which produces extremely heat-resistant spores which are difficult to remove from UHT plants (Hammer *et al.*, 1996). More recently, *Paenibacillus lactis*, another highly heat-resistant mesophilic sporeformer, was isolated from UHT milk (Scheldeman *et al.*, 2004).

Flavour continues to be a major factor inhibiting the wider acceptance of UHT milk in countries where it is not the major form of drinking milk. Various approaches being considered include active packaging which may 'scalp' undesirable flavour compounds or scavenge oxygen (Perkins *et al.*, 2007), addition of flavonoids (Schamberger and Labuza, 2007) and the use of various thermal technologies such as direct steam heating at very high temperatures for very short times (Huijs *et al.*, 2004), use of small-diameter tubular systems

(Kiesner *et al.*, 2004) and forms of electrical and microwave heating with high rates of heating (Montaron *et al.*, 1991; Reznik, 1996; Anon, 2000; Clare *et al.*, 2005). The latter technological approaches are capable of minimising flavour change, while still destroying spores to the desired level, but are less effective in inactivating the native proteinase, plasmin, which can cause bitter flavours during storage of the product (Huijs *et al.*, 2004).

However, a solution to the plasmin problem was devised by considering the reaction kinetics of inactivation of the enzyme. This involved the use of a longer-time, lower-temperature pre-treatment to inactivate the enzyme prior to the high-temperature treatment (Van Asselt *et al.*, 2008). The kinetics-based approach is now being used more widely in computer-based models for predicting the effect of heating profiles on a range of parameters, including destruction of particular organisms, denaturation of proteins, inactivation of enzymes, extent of Maillard reactions, destruction of vitamins, isomerisation of lactose to lactulose, and extent of fouling (Browning *et al.*, 2001; Grijspeerdt *et al.*, 2004; Tran *et al.*, 2008). This computer modelling approach represents a major step forward in predicting the effects of UHT processing, and simulation software for this purpose is now available commercially, e.g. NIZO Premia (Smit *et al.*, 2001).

A further trend in UHT processing is its use in a wide variety of milk-based beverages. These include flavoured milks, sports drinks, and functional foods containing various bioactive ingredients. The challenges associated with these include minimising fouling and gelation during processing, especially for products containing high concentrations of milk proteins or calcium, and stability of the bioactives during processing and subsequent storage.

Some of the shortcomings of UHT processing may, in the future, be overcome by non-thermal technologies. However, to date, no single non-thermal technology capable of producing large quantities of shelf-stable product has been developed (Deeth and Datta, 2002). Combinations of various technologies are often more effective than single technologies (Ross *et al.*, 2003). Thus some technologies used in combination with heat are capable of producing 'sterile' products at temperatures lower than those used in traditional UHT processing. High-pressure processing at high temperature, so-called pressure-assisted thermal sterilisation (PATS) (Ahn *et al.*, 2007), and manothermosonication (Sala *et al.*, 1995) are examples of such combinations.

13.2   UHT processing: definition and principles

UHT processing is generally defined as heating milk between 135 and 145ºC for 1–10 s. In practice, such a range of temperatures and times accounts for most commercial plants (Tran *et al.*, 2008), although some commercial treatments are more severe, operating as high as 152ºC with holding times up to 13 s (Cattaneo *et al.*, 2008). This contrasts with in-container sterilisation of 110–120ºC for 10–20 min, which also produces a sterile product.

While the aim of UHT processing and in-container sterilisation is complete sterilisation of the product, in practice this is not possible. All such products contain some bacterial cells, albeit at low levels. However, the bacterial cells remaining are unlikely to grow under the normal conditions of storage. Such products are referred to as 'commercially sterile'. In general, the bacteria remaining are heat-resistant and thermophilic, which means they have growth temperatures $>~50°C$. There are some unfortunate exceptions to this where bacterial spores are resistant to heat but the organism is mesophilic. The most notable of these, *Bacillus sporothermodurans*, is discussed below.

A basic principle underlying UHT processing is that, for an equal bactericidal effect, a high-temperature, short-time treatment causes less chemical change than a low-temperature, long-time treatment. Given that the chemical changes induced by high-temperature heating include production of, *inter alia*, flavour compounds and brown pigments, this principle provides an explanation for the less-intense heated flavours and brown discoloration in UHT milk compared with in-container sterilised milk. The other important implication of this is that UHT processes which heat milk to a high temperature very rapidly and hold it there for a very short time, even <1 s (Van Asselt *et al.*, 2008), produce a better flavoured product than processes which heat slowly and hold for a longer time (Datta *et al.*, 2002).

A defining feature of UHT processing is the range of temperature–time combinations which can be used. The regulations in some countries set a lower limit of 132°C for 1 s for UHT processing but, as discussed below, such conditions do not ensure an adequately sterilised product. However, 135°C is the lowest temperature a UHT processor would employ, albeit with a holding time of several seconds. This lower limit of heat treatment for UHT sterilisation is generally regarded as the minimal condition necessary to cause a 9-log cycle reduction of thermophilic spores. Kessler (1981) introduced the bacteriological index, $B*$, which has a value of 1 for this condition, which is equivalent to 10.1 seconds at 135°C. Some jurisdictions define the lower limit in terms of the lethality index, Fo, more commonly used for canned foods, where an Fo of 3 minutes is considered approximately equal to a $B*$ of 1. The Fo value is considered to be not as relevant as $B*$ to UHT processing as its reference temperature is 121.1°C (250°F) and it is assumed that the z-value remains the same at UHT temperatures as at this temperature.

The upper limit of temperature–time combinations for UHT processing of milk is dictated by the maximum amount of chemical change to the components of milk. As discussed below, the upper limit has been defined in terms of the destruction of thiamine, vitamin B1; a heat treatment which causes 3% destruction has been defined as the upper severity limit. This is defined as having a chemical index, $C*$, of 1. Thus, the use of $C*$ to define a given heat treatment is based on the kinetics of thiamine destruction. The choice of thiamine for this purpose was obviously arbitrary and, given the fact that thiamine is present at very low levels in milk (~40 μg/litre) and is seldom measured, it can be argued that measuring the change in some other component may be preferable. This is further discussed below.

Therefore, based on the above information, the UHT heating region on a temperature–time graph is defined as the area bounded by a lower line where B^* = 1 and an upper line where C^* = 1 with 135ºC as the lowest acceptable temperature (see Fig. 13.1). The upper temperature limit is largely determined by the capability of the equipment to provide the short holding time necessary to restrict chemical damage to the equivalent of a 3% destruction of thiamine. For most commercial plants this temperature is ~150ºC. However, a recent Dutch invention, innovative steam injection (ISI), is capable of heating milk at temperatures up to 180ºC for a very short time, ~0.2 s (Van Asselt et al., 2008).

Figure 13.1 also shows the temperature–time combinations for in-container sterilisation, which cover the region from ~110 to 120ºC for 10 to 20 minutes. An Fo of 3 minutes, which is equivalent to 3 minutes at 121.1ºC, is the lower limit for sterilisation. It is significant to indicate that the temperature range between in-container sterilisation and UHT, ~120 to 135ºC, is usually used for extended shelf life (ESL) processing (Rysstad and Kolstad, 2006). While the actual heating conditions for ESL are not as well defined as those for in-container sterilisation or UHT, they usually involve a heat treatment for a few

Fig. 13.1   Lines for 9-log reduction of thermophilic spores (B^* = 1), 9-log reduction of mesophilic spores (dotted line) and 3% destruction of thiamine (C^* = 1). UHT conditions are represented by the shaded area.

seconds. In contrast to the sterilisation processes, ESL processing does not ensure 'commercial sterilisation' and the resulting product is not stable at room temperature and must be kept refrigerated.

The preceding discussion refers to holding temperatures and times of the heat treatments and implies that these are the key parameters defining particular heat treatments. This conclusion is also reached in many publications on the topic. This is approximately correct only in the case of systems involving very rapid heating to, and cooling from, the sterilisation temperature, e.g. direct steam heating systems (see below). In these cases the heat input into the product during the heating-up and cooling-down stages is very small compared with the input during the holding stage. In contrast are the systems which involve slow heating to, and cooling from, the sterilisation temperature, e.g. indirect heating systems (see below). In these cases, the heat input over the temperatures which affect $B*$ and $C*$ values, i.e. $>\sim75°C$, relative to that of the holding stage is significant. In a survey of 22 industrial UHT plants (17 indirect, five direct), Tran *et al.* (2008) calculated that the high-temperature holding sections of the indirect and direct plants contribute an average of 47% and 83%, respectively, to the overall $B*$ of the plants and an average of 24% and 61%, respectively, to the overall $C*$ values. These data indicate how misleading it is to only cite the holding temperatures and times. However, on a practical basis, it is much easier to consider only holding temperatures and times as these are routinely recorded; it is much more difficult to determine the overall $B*$ and $C*$ values as the detailed temperature–time profile of the whole plant is seldom known and, even when it is known, some computation is required. This can be facilitated by computer programs such as Excel (Browning *et al.*, 2001; Tran *et al.*, 2008) and commercially available programs such as NIZO Premia (Smit *et al.*, 2001). Therefore, knowledge of the temperature–time profile of a plant is essential if advantage is to be taken of these programs to optimise UHT processing and maximise the quality of UHT products.

13.3   Microbiological aspects

Microbiology is central to UHT processing and products. The UHT process arose out of the need to destroy or exclude microorganisms which are likely to cause milk to spoil during ambient storage. This means that the process has to be severe enough to destroy any microorganisms in the raw product which might grow at room temperature and also has to ensure that in the post-sterilisation section of the plant, i.e. the cooling sections, the aseptic storage tank and the packaging stage, no such microorganisms gain access to the product. This is difficult to achieve 100% of the time and so some UHT milks do contain microorganisms. Many of these are thermophilic spore-forming bacteria, whose spores survive the heat treatment and will not grow below $\sim50°C$, but other types of microorganisms can be present and some can cause spoilage. Estimates of the spoilage rate of UHT packs vary from 1 to 4 per 10,000 (Muir, 1990; Cerf and Davey, 2001) with

a rate of 1 in 10,000 being considered a reasonable commercial standard (von Bockelmann and von Bockelmann, 1998; Robertson, 2003). However, if enrichment procedures are used in the microbiological analysis of UHT milks, the incidence of non-sterility may be found to be much higher than the spoilage rate (von Bockelmann and von Bockelmann, 1998; Coelho *et al.*, 2001).

While the bacterial contaminants in UHT milk can be either heat-resistant spore-formers or post-sterilisation contaminants, most failures are due to post-sterilisation contamination. Published data on commercial UHT plants (Tran *et al.*, 2008; Lewis and Heppell, 2000; Cattaneo *et al.*, 2008) indicate that the heat treatments are in excess of a $B^* = 1$ treatment required for a 9-log reduction of thermophilic spore-formers. However, Cerf and Davey (2001) suggested that non-sterility rates in UHT milk could be explained statistically on the basis of residence time distribution in the UHT plant, whereby a very small percentage of spores pass through the holding tube too fast to be destroyed.

13.3.1   Heat-resistant sporeformers

Several instances of spore-forming bacteria in UHT milk believed to have survived the heat treatment have been reported (Meier *et al.*, 1995; Forschino *et al.*, 1990). The spores capable of surviving the UHT process are mainly *Geobacillus stearothermophilus* (previously *Bacillus stearothermophilus* and before that *Bacillus calidolactis*), *B. subtilis* (Muir, 1990), *B. megaterium* (Hassan *et al.*, 1993), *B. sporothermodurans* (Pettersson *et al.*, 1996; Scheldeman *et al.*, 2004, 2006) and *Paenibacillus lactis* (Scheldeman *et al.*, 2004). *G. stearothermophilus* has a high survival potential with some strains showing extreme heat stability. Decimal reduction times (the time taken for a 1-log reduction) at 121ºC of up to 6.2 minutes have been reported (Burton, 1988). Intaraphan (2001) found that the spores of one strain of *G. stearothermophilus* isolated from a farm hot water supply survived a laboratory heat treatment of 154ºC for up to 9 s. However, being a strict thermophile, this organism is unable to grow below ~50ºC and hence presents as a major problem only in hot climates or temperature-abused samples. As it produces acid but no gas, it has been associated with the 'flat sour' defect in sterile products (Gilmour and Rowe, 1990; Kalogridou-Vassiliadou, 1992).

The discovery of *B. sporothermodurans* in milk is comparatively recent (Pettersson *et al.*, 1996). It is extremely heat-resistant, with heating conditions required for its inactivation being 148ºC for 10 s or 150ºC for 6 s (Hammer *et al.*, 1996). Unlike *G. stearothermophilus*, it is a mesophile, i.e., able to grow at room temperature. Fortunately, *B. sporothermodurans* does not appear to cause spoilage other than a slight pink discoloration of the milk and seldom reaches counts of greater than 10^5/mL. Once this organism has contaminated a plant, it is difficult to remove and has caused the closure of some UHT plants (IDF, 2000). One cause of its presence in UHT milk is believed to be the practice of reprocessing of out-of-date UHT milk containing spores of the organism (Pearce, 2004).

Scheldeman *et al.* (2004) analysed individual packages of UHT milk produced during an 'obstinate contamination of a dairy company'. After preheating the milk at 100ºC for 30 minutes to deliberately select for highly heat-resistant spores, they found two species which they identified as *B. sporothermodurans* and *Paenibacillus lactis.* This was the first time *Paenibacillus* had been isolated from UHT milk, although *Paenibacillus* spores had been previously reported to survive heating at 120ºC. *Paenibacillus* spores have been isolated from silage and feed concentrates, which may be the origin of the organism in milk. From this work, such organisms may be expected to be present in milks throughout the world but they have seldom been targeted.

13.3.2   Post-sterilisation contaminants

Any microorganism in UHT milk which is not a heat-resistant spore-former must enter the product after the sterilisation step, assuming the appropriate UHT heating conditions are used. In addition, some spore-formers may also be post-sterilisation contaminants. Since *Bacillus* species make up the majority of the contaminants (Lück *et al.*, 1978; Forschino *et al.*, 1990; Skladal *et al.*, 1993; von Bockelmann and von Bockelmann, 1998; Coelho *et al.*, 2001), it is possible that many of these are post-sterilisation contaminants. One source of these organisms is the seals in the homogeniser (if downstream of the sterilisation section). Kessler (1994) showed that spores under seals exhibited high heat resistance, due to the very low water activity in that microenvironment, and could act as a reservoir of contaminating spores. This contamination can be minimised by frequent changing of these seals. Simmonds *et al.* (2003) found that spores such as those of *G. stearothermophilus* acquired significantly enhanced heat resistance when attached to stainless steel. This may explain why some spores, which usually have low heat resistance, appear to survive high-temperature treatment in UHT plants.

Non-spore-forming bacteria, both Gram-positive and Gram-negative, have also been isolated from UHT milk in several studies. In a South African study, 12% of the non-sterile UHT milks contained non-spore-forming bacteria (Lück *et al.*, 1978) and in a Brazilian study 7% of the microorganisms isolated from UHT milks were non-spore-formers (Coelho *et al.*, 2001). The bacteria isolated include *Micrococcus* and *Corynebacterium*-like Gram-positive bacilli (Coelho *et al.*, 2001), *Streptococcus lactis* and unspecified Gram-negatives (von Bockelmann and von Bockelmann, 1998) and *Staphylococcus aureus*, *Sc. faecalis* and *Enterobacter sakazakii* (Skladal *et al.*, 1993). The presence of these organisms in a product that is supposed to be commercially sterile emphasises the constant battle that UHT processors have to maintain sterility in the whole processing and packaging line.

A filamentous fungus, *Fusarium oxysporum*, has also been isolated from defective UHT milks produced in several countries. It causes a cheesy flavour, a ropey texture and often excessive gas, which causes packages to become swollen during storage (A. Hocking, pers commun., 2005). The fungus originates from plants and soils, and can contaminate the air inside the filling machine if a

positive pressure is not maintained. It can grow at low oxygen levels and grows well at room temperature. In aseptic fillers, it can grow behind plastic and rubber parts such as seals and gaskets, and once the fungus has seeded the equipment, it is very difficult to remove (K. Scrimshaw, pers commun., 2004).

13.3.3   Raw milk quality

Raw milk quality is of utmost importance for the quality of UHT milk. The probability of spore-formers surviving UHT treatment increases if the raw milk contains a high level of highly heat-resistant spores. It has been postulated that there is a direct relationship between the spore count of the raw milk and the incidence of bacteriological defects developing after sterilisation, i.e., the higher the numbers of spores present in raw milk, the higher the percentage of sterilised samples developing faults (Waes, 1976).

   Another important relationship between the quality of UHT milk and that of the raw milk is through the survival of heat-resistant proteinases and lipases produced during growth of psychrotrophic bacteria such as *Pseudomonas*, *Alcaligenes* and *Flavobacterium* in the raw milk. Some of these enzymes have much greater heat resistance than highly heat-resistant spores. Marshall (1996) found that these enzymes remained active following a heat treatment of 149ºC for 10 s, while Muir (1996) reported that proteinases of fluorescent pseudo-monads had residual activities ranging from 14 to 51% at 140ºC for 5 s. Quite low levels of residual proteinase can cause a reduction of shelf-life of UHT milk by causing bitterness and gelation. In fact, Adams *et al.* (1975) suggested that the amount of proteinase needed to limit the shelf-life of UHT milk can be easily synthesised within a day by some high-proteinase-producing bacteria.

   UHT milk prepared from raw milk containing more than 5×10^6 cfu/mL psychrotrophs is at risk of spoilage due to heat-resistant enzymes (Varnam and Sutherland, 2001). While this is a good guide for UHT processors, it is not possible to predict from the total bacterial count whether a milk will contain a significant amount of proteinase as each mix of bacteria has a different propensity to produce proteinase. Counts as low as 10^5 cfu/mL can produce noticeable quantities but some milks with 10^7 cfu/mL do not contain noticeable levels (Haryani *et al.*, 2003).

13.4   UHT processing: methods and characteristics

There are several different methods of UHT processing. The most common heating media are steam and superheated water, but electrical heating can also be used.

13.4.1   Traditional steam and hot-water systems

UHT processes are generally characterised as direct or indirect. *Direct* processes use steam which is mixed directly with the milk. It may be mixed by injecting

steam into a stream of flowing milk (known as *steam injection*) or alternatively milk can be sprayed into a chamber of steam (known as *steam infusion*). *Indirect* processes are characterised by a barrier between the heating medium and the milk through which the heat is transferred. In this case, the heating medium, either steam or hot water, does not come into contact with the milk. Heat exchangers used in indirect UHT plants can be of either tubular or plate design. Because of the different method of heat transfer, indirect heating is considerably slower than direct heating. In direct heating, steam gives up its latent heat to the milk almost instantaneously and is converted to water.

In practice in a UHT plant, milk is heated from refrigeration temperature of ~5°C to sterilisation temperature of ~140°C and, after holding at this temperature for a few seconds, is cooled to ambient or lower before being aseptically packaged. On the heating side, milk is always pre-heated indirectly up to an intermediate temperature of 70 to 95°C. It is the heating from this temperature to the sterilisation temperature which can be direct or indirect heating and which characterises the plant as 'direct' or 'indirect'. The cooling side also differs for these two types of plant. In direct plants, the water introduced by the condensing steam is removed under vacuum and this also cools the milk. If the vacuum level is set correctly, the same amount of water introduced is removed and the temperature of the milk is reduced to approximately the pre-heat temperature.

In accordance with the basic principle of UHT processing discussed above that, for an equal bactericidal effect, a high-temperature, short-time treatment causes less chemical change than a low-temperature, long-time treatment, direct plants in general cause less chemical change than indirect plants (Datta *et al.*, 2002). The differences in these chemical changes are discussed below. Conversely, higher temperatures can be used in direct plants than indirect plants without causing excessive chemical change. This is particularly important for destroying highly heat-resistant spores.

A major operational difference between indirect and direct plants is the ability to recover heat used in heating the milk. In indirect plants a large proportion (>90%) of heat is recovered by using the hot milk to heat the incoming cold milk. Thus the hot milk flows through the heat exchanger in the opposite direction to the flow of cold milk on the other side of the stainless steel barrier of the heat exchanger. The countercurrent flow is used to minimise the temperature differential between the hot and cold milks. With direct plants, heat is not recovered from the vacuum cooling step but only from the indirect cooling stages after the vacuum cooling step. Typically heat recovery in a plant with direct heating is ~50%.

From the above, it is apparent that a range of choices is available to the UHT processor who has to take account of three major issues: the percentage heat recovery, which affects the efficiency of the operation, the extent of chemical change to tolerate, which primarily affects the taste of the milk, and the extent of bacterial destruction, which mainly concerns highly heat-resistant spores. In order to address these three issues, two major manufacturers of UHT equipment, APV and Tetra Pak, developed indirect–direct combination processes, which

have a percentage heat recovery between conventional direct and indirect plants, and use direct heating to enable high sterilisation temperatures to be attained without causing excessive chemical damage. In the APV *High Heat Infusion* system, the vacuum cooling chamber is placed after the preheat and before the direct heating to sterilisation step; this enables all the heat in the hot milk after the sterilisation step to be recovered. The Tetra Pak *Tetra Therm Aseptic Plus Two* combination system operates like a standard direct plant up to the steriliser holding tube but then the heat is recovered from the hot milk by using it to heat the cold milk in the preheat section until the sterilised milk is cooled to ~90ºC, after which it is vacuum cooled to remove the water added during the direct sterilisation heating step (Bake, 1997).

An integral step in most UHT plants is homogenisation, which is designed to reduce the size of the fat globules to <1 micron so that little, if any, fat rises to the top of the milk during storage, which can be up to 12 months at room temperature. Homogenisation is usually performed in a two-stage homogeniser in which the first stage is set at ~20 MPa and the second is set at ~5 MPa. The second stage is designed to disperse any clumps of fat globules which may form after the first homogenisation valve. It is customary to place the homogeniser downstream, i.e., after the sterilisation step in direct plants and either upstream or downstream in indirect plants. Direct heating can cause the formation of aggregates of protein, which impart an astringency to the milk; homogenisation disperses these aggregates and improves the flavour of the milk. Practically, it is preferable to have the homogeniser placed upstream as in that position it does not have to be sterile. This is significant as the homogeniser, when placed downstream, is a common source of bacterial contamination as mentioned above.

13.4.2   Electrical heating

An alternative tubular heating system used in UHT processing of milk is based on direct electrical heating of the stainless steel tube. In this process, the stainless steel tube carrying the milk acts as an electrical resistor and is heated by the passage of an electrical current by what is known as the Joule effect. The heat is then transferred to the circulating product from the surface of the tube by conduction and mixed convection (Lefebvre and Leuliet, 1997). While the generic name given to the technology is Current Passage Tube, or Tube à Passage de Courant (TPC) in French, it is also referred to as Actijoule®, the brand name of one of the companies manufacturing the equipment. It was developed in France in the late 1980s and commercialised in the early 1990s (Deeth, 1999).

Important features reported for this technology are the linear rise in temperature of the product; the small and constant temperature differential between the tube and the product; a variable heating flux rate for different products, enabling fast heating for low-viscosity products such as milk; and absence of thermal inertia in the system for start-up and shut-down – heating

ceases when the power is switched off (Montaron *et al.*, 1991). Montaron *et al.* (1991) reported a system for production of UHT milk which included electrical heating from 100 to 140ºC in 4 seconds, a temperature differential between the tube and the milk of 10–15ºC and energy efficiency of ≥95%. The equipment was capable of achieving temperatures up to 170ºC, if required.

13.4.3   Temperature–time profiles

Temperature–time profiles for commercial direct and indirect plants using steam or hot water are shown in Figs 13.2 and 13.3, respectively. The immediately noticeable differences are the much faster heating and cooling in the high-temperature region and the much shorter overall time for the direct plant than the indirect plant. These differences are typical of commercial plants; however, the profiles of both types of plants vary considerably as reported by Tran *et al.* (2008) and Lewis and Heppell (2000). Some indirect plants feature faster heating rates in the high heat section than that shown in Fig. 13.3; such plants may involve plate heat exchangers or tubular heat exchangers in which large-diameter pipes have been replaced with bundles of pipes of much smaller diameter, e.g. 6–10 mm. While the latter facilitate faster heat exchange, they are also more susceptible to fouling and, in extreme cases, blockage.

Fig. 13.2   Temperature–time profile of a commercial direct UHT plant.

Fig. 13.3   Temperature–time profile of a commercial indirect UHT plant.

The profiles in Figs 13.2 and 13.3 are shown with linear changes in temperature whereas in reality some will be curved. For countercurrent flow, the shape depends on the relative flow rates and specific heats of the product and heating/cooling medium. Thus the changes are close to linear in the regeneration sections and approach linearity when water is the heat exchange medium and milk is the product. The largest deviation from linearity occurs when steam is the heating medium (Lewis and Heppell, 2000).

The profile for the electrically heated plant as reported by Montaron *et al.* (1991) shows quite rapid heating from the preheat temperature to the sterilisation temperature, reflecting operation of a plant at a high heating flux rate. In this plant, temperature changes in each section would be expected to be very close to linear. Because of the advantages of rapid heating in the sterilisation section for minimising heat-induced chemical changes, combined with the capacity to reach temperatures of $\geq 150°C$ to kill heat-resistant bacterial spores, plants with rapid heating have been sought. For this reason, ohmic heating (Reznik, 1996; Anon, 2000) and microwave heating (Clare *et al.*, 2005) have considerable potential to be used for UHT processing of milk.

13.4.4   Residence time distribution

When considering the amount of heat received by milk in UHT processing, it is often assumed that all particles in the milk receive the same heat treatment as they move through the plant. This is particularly relevant to the holding tube where the temperature and the time taken for the milk to pass through the section are usually used to define the heat treatment. In practice, however, the particles in the milk take a range of times to pass through the holding tube; the spread of

these times is known as the residence time distribution (RTD). In UHT plants, the residence time of the fastest particle in the holding tube can be much less than (e.g. half) that of the average or mean residence time, the nominal time cited. However, if the RTD is quite narrow, the residence time of the fastest particle will be not much less than that of the mean residence time.

The RTD depends on the nature of the flow of the product through the holding tube, which can be described by the Reynolds number, Re, a dimensionless number given by the formula $Re = \rho v d / \mu$, where $\rho = $ density, $v = $ velocity, $d = $ diameter of the pipe and $\mu = $ viscosity. A low Reynolds number is associated with low-density, high-viscosity products, and narrow tubes. Such conditions give rise to laminar flow and a broad residence time distribution. Conversely, a high Reynolds number is associated with turbulent flow and a narrow residence time distribution. The Reynolds number for laminar flow is <2000 and for turbulent flow >4000. A Reynolds number between 2000 and 4000 is characteristic of transitional flow, which has characteristics of both laminar and turbulent flow. The flow characteristics can be improved by features that disrupt streamline flow, such as bends or baffles in the tube, which are special tube designs that enhance turbulence. The flow through plate heat exchangers is more turbulent than through tubular heat exchangers and holding tubes, and the flow through larger pipes is more turbulent than through narrow tubes. This has implication for pilot-scale equipment, which typically has tubes of ≤10 mm diameter (Tran *et al.*, 2008).

A practical consequence of a broad residence time distribution is that some bacterial spores may traverse the holding tube very quickly. This is unlikely to be a safety issue for milk as the heat resistance of vegetative pathogens is much lower than a $B* = 1$ heat treatment, and the presence of the major spore-forming pathogen, *C. botulinum*, is extremely unlikely (Codex Alimentarius, 2004). However, a broad residence time distribution may be a cause of spoilage (Cerf and Davey, 2001), as discussed above.

13.5   Changes in milk during UHT processing

The high heat treatment in UHT processing causes several changes which affect processing efficiency and product quality. The extent of these changes is largely dependent on the nature of the heating, particularly the temperature–time profile and the nature of the product. Fouling or deposit formation is the most important processing effect, while whey protein denaturation and subsequent formation of protein complexes involving denatured β-lactoglobulin, lactose–protein interaction and subsequent Maillard reactions, and isomerisation of lactose to lactulose are the most significant changes affecting product quality.

13.5.1   Whey protein denaturation and formation of protein complexes
Denaturation of the whey proteins, particularly β-lactoglobulin and, to a lesser extent, α-lactalbumin, is one of the most important heat-induced changes. It

commences at temperatures as low as 40°C but is largely reversible up to ~70°C (Iametti and Bonomi, 1996). Denaturation of β-lactoglobulin involves firstly dissociation of its very compact dimeric structure and then unfolding of the globular monomer. The latter step exposes an active free sulphydryl group which is normally buried inside the globular structure. This –SH group, or another resulting from intramolecular sulphydryl–disulphide reactions (Creamer *et al.*, 2004), interacts with other –SH groups or, more commonly disulphides of cystines, in intermolecular sulphydryl–disulphide reactions. These reactions occur between molecules of β-lactoglobulin, but also significantly between β-lactoglobulin and κ-casein, and between β-lactoglobulin and α-lactalbumin.

The extent of whey protein denaturation is greater for indirect plants than for direct plants. Tran *et al.* (2008) showed that, based on the kinetics of denaturation reported by Dannenberg and Kessler (1988), commercial indirect plants cause almost complete denaturation of β-lactoglobulin but the direct plants cause only 74–92% denaturation. Since α-lactalbumin is more heat resistant than β-lactoglobulin, the predicted levels of its denaturation, based on the reaction kinetics data of Lyster (1970), for the indirect and direct plants were considerably lower and showed a wider spread: 25–90% for indirect and 27–58% for direct plants. Because the heat sensitivity of α-lactalbumin is lower than that of β-lactoglobulin, and because of the spread of denaturation levels for commercial plants, α-lactalbumin denaturation has been suggested as a measure of heat-induced chemical change in UHT plants (Tran *et al.*, 2008). While undenatured α-lactalbumin can be readily measured by HPLC (Elliott *et al.*, 2005), its use as a chemical heat index is not ideal, as the level of α-lactalbumin in raw milk shows considerable variation and hence the extent of denaturation cannot be determined.

13.5.2   Fouling or deposit formation

During UHT processing of milk, a deposit builds up on the heat exchanger, which reduces the operational efficiency of the plant as the deposit acts as a partial insulator and reduces the rate of heat transfer to the milk. The run-time before cleaning is required varies with the type of UHT plant. Indirect plants foul more quickly than direct plants with the fastest fouling occurring in plants with plate heat exchangers. Approximate run times for plate indirect, tubular indirect and direct commercial UHT plants are 8–12, 12–16 and >~18 hours, respectively. Although the flow of milk in a plate heat exchanger is turbulent and this tends to minimise fouling, the flow channels are narrow and hence block more readily than tubular heat exchangers. In general, increasing the flow rate of milk through UHT plants, thus increasing the Reynolds number, decreases the rate of fouling (Belmar-Beiny *et al.*, 1993).

The nature of the deposit varies throughout the plant as it depends on the temperature of the heat exchanger. In general, two broad types of deposit are identifiable (Burton, 1988). One, known as type A, occurs at the lower temperatures in a UHT plant, up to ~110°C; it is quite voluminous and consists

largely of protein. The other, type B, which occurs at temperatures >~110ºC, is hard and compact and consists largely of minerals, principally calcium phosphate. A major effect of the former is blocking the flow of milk and causing an increase in back-pressure, while the major effect of type B deposits is decreasing heat transfer from the heating medium to the product. The latter causes a decrease in the temperature of the heated product, which, in commercial plants, causes more heat to be supplied to the heating medium. This effectively exacerbates the fouling as the rate of deposit formation increases with an increase in temperature differential between the heating medium and the product. Ultimately, the deposit builds up to a point where the plant has to be shut down and the deposit removed. Removal of the deposit is achieved by washing with alkali to remove the organic material and with acid to remove the inorganic material.

Type A deposit is largely attributable to the heat denaturation of β-Lg. The first stage of denaturation, when the native protein unfolds exposing a previously buried –SH group, is critical to fouling, as it has been shown that the longer the unfolded stage of β-Lg is present in the UHT plant, the greater the deposit formation or, conversely, the shorter the unfolded form is present, the longer the run-time of the plant (Grijspeerdt *et al.,* 2004). This suggests that the unfolded form is 'sticky' and readily attaches to the walls of the stainless steel tube and forms a base for deposit formation. Thus, the temperature controlling the formation of the reactive β-Lg in milk, and its subsequent interactions with other 'activated' β-Lg molecules and milk proteins containing disulphide linkages, is critical. Preheating at 65ºC was shown to cause much more fouling of concentrated reconstituted skim milk than preheating at 95ºC (Prakash, 2007). By contrast, Srichantra *et al.* (2006) investigated the effects of milk preheating on fouling of UHT sterilising plants by recombined whole milks and found that fouling rates increased with the severity of the pre-heat treatment. They concluded that preheating of previously homogenised whole milks exacerbates fouling.

Type B deposit consists largely of calcium phosphate whose solubility decreases at high temperature, causing it to precipitate onto the surface of the heat exchangers. The calcium is believed to form bridges between the adsorbed β-Lg on the heat exchanger wall and the aggregates formed through the interactions of β-Lg with other whey proteins and caseins. This tends to compact the spongy protein base deposit and impede the transfer of heat from the heating medium to the milk.

Casein deposits on the metal surface either by associating with reactive β-Lg molecules, as micellar casein (Jeurnink *et al.*, 1996), or as part of coagulated casein brought about by aggregation of casein micelles. Casein aggregation does not appear to be a major contributor to fouling in single-strength cows' milk at the normal pH, i.e. ~6.7, and at normal UHT temperatures, i.e. 138–145ºC. However, in certain circumstances, such as concentrated milk, milk with high ionic calcium and/or pH < 6.6, and heating at high UHT temperatures, e.g. ~150ºC, casein aggregation can be a major cause of fouling. High ionic calcium

levels can cause severe fouling. This is particularly evident in goats' milk in which ionic calcium levels are much higher than in most cows' milks. It is generally not possible to UHT process goats' milk without reduction of ionic calcium by addition of citrate or phosphates or use of cation-exchange resins (Prakash, 2007; Boumpa *et al.*, 2008). It is of interest that the conditions under which casein aggregation contributes significantly to fouling are those in which the heat stability of milk is low.

13.5.3   Maillard reactions

Maillard reactions are initiated during UHT heating but also continue during storage. They are a complex series of reactions which commence with the interaction of lactose and the ϵ-amino acid of the side chain of lysine in milk proteins, progress with the formation of several intermediate compounds such as hydroxymethylfurfural (HMF), formic acid and methyl ketones, and conclude with formation of advanced Maillard products such as galactosyl-β-pyranone (Cattaneo *et al.*, 2008) and the brown-coloured melanoidins.

One of the first Maillard products formed is lactulosyl-lysine. On hot acid treatment this is degraded to furosine which is widely used as a chemical index of the severity of heating (Resmini and Pellegrino, 1994). HMF is also used as a heat index but suffers from the disadvantage of being both formed and degraded during the progress of the Maillard reaction (Elliott *et al.*, 2005). Methyl ketones were recently proposed as an index of heat treatment of milk; however, they can also arise during storage from oxidation of lipids (Perkins *et al.*, 2005). Formic acid produced by the Maillard reaction contributes to the decrease in pH during heat treatment. The advanced Maillard products are only observed after very severe UHT heating (Cattaneo *et al.*, 2008) and hence brown discoloration of normal milk during UHT heating is not usually observed. However, it may be observed in modified milks such as lactose-hydrolysed milks in which Maillard reactions are enhanced (Messia *et al.*, 2007).

13.5.4   Lactose isomerisation

Isomerisation of lactose (galactose–glucose) to lactulose (galactose–fructose) occurs during heat treatment and is positively correlated with the severity of heat treatment. It has also been shown to correlate positively with the intensity of the cooked flavour in UHT milk (Burton, 1988).

Lactulose is considered to be the most reliable index of heat treatment of UHT milk, as it is not present in unheated milk and it changes little during storage of the milk (Elliott *et al.*, 2005). In UHT milk it typically ranges from 90 to 250 mg/L in directly heated milk and from 310 to 570 mg/L in indirectly heated milk (Calvo *et al.*, 1987). It has been proposed that the lactulose content of UHT milk should be <600 mg/L to enable it to be distinguished from sterilised milk, which should have >600 mg/L of lactulose (EU Directive 92/46, September 1995).

Lactulose is now regarded as a functional ingredient as it is not digested or absorbed in the human gut. Because of these properties it is approved as a laxative in some countries. It also stimulates the growth of bifidobacteria (de Block *et al.*, 1996).

13.6   Changes in UHT milk during storage

13.6.1   Gelation

Gelation during storage is a major defect which limits the shelf-life of UHT milk (Datta and Deeth, 2001). It is characterised by a gradual increase in viscosity followed by a quite rapid increase in viscosity and formation of a custard-like gel. The gel is a three-dimensional protein network whose formation is irreversible.

The mechanism of gelation in single-strength milk is not fully understood but is usually associated with proteolysis. According to McMahon (1996), proteolysis of caseins causes the release of κ-casein–β-lactoglobulin complexes from the casein micelle into the surrounding milk serum, and when the concentration of the $\kappa\beta$-complexes in solution reaches saturation the protein network forms a gel. Gelation of concentrated UHT milk appears to proceed by a different mechanism as it does not appear to be related to proteolysis.

Proteolysis of the caseins can be caused by either the native milk proteinase, plasmin, or heat-resistant bacterial proteinases produced by psychrotrophic bacterial contaminants in the milk before heat treatment. These two enzymes have different actions on casein, with plasmin targeting β- and α_s-caseins while the bacterial enzymes generally show preference for κ-casein. A major significant difference between the two proteinase types is that plasmin produces large hydrophobic peptides, while the bacterial enzymes produce smaller more hydrophilic peptides. These peptides show different acid solubilities and reversed-phase (RP) HPLC behaviour, making them readily differentiated (Datta and Deeth, 2003). Those from bacterial proteinases are soluble in TCA (4%) and elute early in RP-HPLC, while the peptides from plasmin proteolysis are soluble at pH 4.6 but insoluble in 4% TCA, and have much longer retention times in RP-HPLC. These different behaviours enable the cause of proteolysis to be determined.

Several factors affect the time to gelation during storage. The more severe the heat treatment the longer the time to gelation, i.e., total heat input over all time–temperature combinations. Thus, for the same bactericidal effect, indirectly heated milks take longer to gel than directly heated milks. Two explanations for the effect of heat severity are that the greater the severity of heat treatment, the more inactivation of the proteinases, and the less accessibility of heated proteins to proteinases. The temperature of storage also affects the time of onset of gelation but in a rather curious manner. Storage at low (~4ºC) and high (~35–40ºC) temperatures delays onset of gelation but storage at ~25–30ºC is optimum for gel formation. One explanation for this phenomenon is that covalent

crosslinking reactions occur between proteins at the higher storage temperatures (see below) and hold the micelle intact and prevent the release of the $\kappa\beta$-complex into the medium (Henle *et al.*, 1996; McMahon, 1996).

Gelation can be controlled through processing to ensure a minimum level of active plasmin, by ensuring raw milk quality is high in terms of both mastitis and psychrotroph counts, and by use of additives such as sodium hexametaphosphate. Newstead *et al.* (2006) demonstrated that a pre-heat treatment of at least 90ºC for 30 seconds was necessary to inactivate milk plasmin and prevent proteolysis of directly processed UHT milk during storage. In a study of UHT milks in Turkey, Topçu *et al.* (2006) found that UHT milk processed at 150ºC rather than 140ºC showed less proteolysis and took longer to gel during storage. Furthermore he showed that UHT milk manufactured from low-quality raw milk, which had high bacterial and somatic cell counts, was much more prone to gelation and development of bitterness during storage than that made from good quality milks. Sodium hexametaphosphate or polyphosphate can be added to UHT milk to retard gelation (Kocak and Zadow, 1985). Interestingly it does not prevent proteolysis occurring. Addition of polyphosphates is particularly useful for delaying gelation in UHT concentrated milks (Leviton *et al.*, 1963).

13.6.2   Protein changes

The proteins in UHT milk change considerably during storage. This is most apparent in their behaviour on HPLC. Gaucher *et al.* (2008) presented chromatograms of semi-skimmed UHT milk after storage at 4, 20 and 40ºC for 180 days. They showed that the sharp peaks of the major caseins in freshly processed milks broadened and merged into each other in the stored milks; this was most marked for the samples stored at the higher temperatures where it became impossible to distinguish individual caseins. The authors attributed the changes to Maillard reactions and proteolysis, and possibly other chemical reactions such as dephosphorylation and disulphide-based polymerisation.

The most obvious changes are proteolysis, which causes bitterness and gelation, and Maillard reactions, which cause brown discoloration. The latter are particularly noticeable in samples stored at 40ºC. The first step in the Maillard reaction, lactosylation, is evident in 2-D polyacrylamide gel electrophoresis (PAGE) where the different levels of derivatisation of the individual proteins present as a series of spots with molecular weights differing by the molecular weight of lactose (Dr Raj Gupta, pers. commun.).

A vast array of peptides can be formed by proteolysis. For example, Gaucher *et al.* (2008) identified 181 peptides in stored UHT milk using mass spectrometry. These authors showed that, through knowledge of the preferred cleavage sites of the different proteinases, it was possible to attribute the peptides to the action of particular proteinases. In this way they were able to identify peptides resulting from the action of plasmin and bacterial proteinases, but also several from the action of cathepsins G, B and D as well as elastase.

Deamidation of proteins occurs during storage of UHT milk, particularly at elevated temperatures. This can be seen in 2-D PAGE electrophoretograms, with separations based on charge and size, where additional spots corresponding to proteins with additional negative charges are observed. The deamidation reaction converts asparagine to aspartic acid. Because different levels of deamidation occur on particular proteins, several different protein species can result from one parent protein. This is apparent in the 2-D PAGE gels in Fig. 13.4, which shows an increase in the number of spots for individual proteins in UHT milk stored at 40°C for 2 months. For example, the α_{s1}-casein pattern changes from one with one dominant spot to one with at least five distinct spots (Gupta *et al.*, 2007).

While protein crosslinking via disulphide bonding occurs between the proteins containing cysteine or cystine residues, other covalent crosslinks develop, which result in the formation of oligomeric proteins which are not reduced when run on SDS-PAGE under reducing conditions. On 1-D gels they appear as broad bands with molecular weights higher than all monomeric caseins; on 2-D gels they appear as a broad smear as they consist of many

(a) Raw Milk (Red) IPG 4-7

(b) D30B4_2M (Red) IPG 4-7

(c) D30B28_2M (Red) IPG 4-7

(d) D30B40_2M (Red) IPG 4-7

Fig. 13.4   Reducing 2-D electrophoretograms of raw milk (a) and UHT milk stored at 4°C (b), 28°C (c) and 40°C (d) for 2 months. The UHT milk was directly processed using steam infusion at 138°C for 6 s with a 30 s preheat hold. The first dimension of the 2-D PAGE gel was run under isoelectric focusing on a pH 4–7 strip while the second dimension was run with SDS. 1 = α_{s1}-casein; 2 = α_{s2}-casein; 3 = β-casein; 4 = κ-casein; 5 = β-lactoglobulin; 6 = α-lactalbumin; 7 = crosslinked proteins. (Gupta, Holland, Deeth and Alewood, unpublished).

different protein species (Figs 13.4c and d). This crosslinking is due to the reaction between dehydroalanine and amino acids such as lysine and histidine on adjacent protein molecules (Henle *et al.*, 1996). The extent of crosslinking can be monitored by measuring lysinoalanine (LAL) in acid-hydrolysed proteins. LAL has been shown to form during severe UHT heating (Cattaneo *et al.*, 2008) and increase during storage of UHT milk, especially at elevated temperatures such as 45ºC (Al-Saadi and Deeth, 2008).

13.6.3   Flavour

UHT milk immediately after production has a sulphury flavour and is charac-terised by an array of volatile sulphur compounds including hydrogen sulphide, methanethiol, dimethyl disulphide, dimethyl sulphide, carbon disulphide, carbonyl sulphide, dimethyl trisulphide, dimethyl sulphoxide and dimethyl sulphone. Of these, methanethiol, dimethyl sulphide and dimethyl trisulphide have been shown to contribute most to the sulphurous note (Vazquez-Landaverde *et al.*, 2006). The concentrations of all these compounds decrease during storage with the result that after a few days the initial strong cooked flavour disappears. The flavour remaining after that time is referred to as heated or sterilised and is due to residual sulphur compounds together with products of the Maillard reactions, such as diacetyl, lactones (C8, C10, C12), methyl ketones (C5, C7, C9, C11), maltol, vanillin, benzaldehyde and acetophenone. After a period of optimum flavour which, at a storage temperature of 25ºC, is from about two to five weeks after production, oxidation of lipids occurs, which causes an increase in aliphatic aldehydes and methyl ketones (Perkins *et al.*, 2005) and a resultant stale flavour, considered by some to be the major flavour defect in stored UHT milk (Wadsworth and Bassette, 1985).

The oxygen content of UHT milk has a significant effect on its flavour. It affects firstly the rate at which the initial sulphury, cooked flavour disappears and secondly the rate of development of stale flavours. Without taking into consideration any oxygen permeating through the packaging material, the major determinants of oxygen levels are the UHT processing method and the type of packaging. Direct UHT processing, in which water is removed from the milk by vacuum after sterilisation, reduces the oxygen content to <1 mg/L, while milk produced by indirect processes is virtually saturated with oxygen at 7–9 mg/L, assuming that a deaeration step is not used (Datta *et al.*, 2002). While the oxygen content of directly processed UHT milk may be very low immediately after manufacture, it does not necessarily remain low as it can be affected by storage in the aseptic tank (if not nitrogen-blanketed) prior to packaging and in the final package. The length of time the milk remains in the aseptic tank largely determines whether the milk becomes saturated before packaging. The amount of headspace in the final package determines whether the milk becomes saturated with oxygen during storage. In three commonly used UHT packaging systems, paperboard cartons formed and filled during packaging (e.g. Tetrabrik cartons), pre-formed paperboard cartons filled and closed during packaging (e.g.

Combibloc cartons) and plastic bottles filled and closed during packaging, sometimes blown on line, the headspace in one-litre packs has been shown to contain 7–8 mL, 21–40 mL and 55–63 mL, respectively (Perkins *et al.*, 2005). Thus, milk in the last two types of pack remains virtually saturated with oxygen during storage and this will affect the amount of oxidation products, such as aldehydes and methyl ketones, produced during storage. Packaging material which absorbs oxygen has been shown to reduce the production of these compounds during storage (Perkins *et al.*, 2007).

13.6.4   Fat separation

Most UHT whole milks develop a fat layer during storage but this layer should not represent a significant proportion of the fat. However, substantial fat separation can occur if the average fat globule size is too high or the milk contains some large globules. The average fat globule size should be ~0.7 microns with a maximum of 1–2 microns (von Bockelmann and von Bockelmann, 1998). In one study the fat layer thickness in UHT milk was found to correlate well with the number of fat globules in the size range 1.5–2.0 microns (Hillbrick *et al.*, 1998). Industrially, a common specification for the size of the fat globules is not more than 2% > 5 microns and not more than 5% > 2 microns. This is based on the fact that it is only the large fat globules which rise to the top of the milk and cause a cream layer. It can be shown that if only a small proportion of fat globules have diameters greater than ~2 microns, the amount of fat contained in those large globules, and hence which will separate during storage, can represent a substantial proportion of the total amount of fat (Hillbrick *et al.*, 1998). Some large globules can pass through the homogeniser if the valves are not well maintained and become damaged.

Hardham *et al.* (2000) compared the effects of high-pressure micro-fluidisation and conventional valve homogenisation during UHT processing on fat separation during storage. They found only slight fat separation in the microfluidised samples after 9 months of storage at 25ºC, compared with moderate fat separation after 2–3 months of storage at the same temperature for milk subjected to conventional valve homogenisation. The improved stability of the microfluidised samples may be due to the fact that the fat globule size in these samples was about 25% smaller than that in the homogenised milk.

13.7   Sources of further information and advice

Further information can be obtained from monographs focused on UHT processing, the major ones being IDF (1981), Burton (1988), von Bockelmann and von Bockelmann (1998) and Lewis and Heppell (2000), chapters in textbooks such as Tetra Pak (1996), Datta and Deeth (2005), Kelly *et al.* (2005), Manzi and Pizzoferrato (2006) and Robertson (2005), and chapters in the *Encyclopedia of Dairy Sciences* such as Deeth and Datta (2003), Hinricks and

Rademacher (2003) and Robertson (2003). Other sources of collated information are reviews such as those by Kosaric *et al.* (1981) on UHT milk, Muir (1984) on UHT milk concentrate, Calvo and de la Hoz (1992) on heated milk flavour, IDF (1996b) on UHT cream, Cunha (2001) and Datta and Deeth (2001) on age gelation, Datta *et al.* (2002) on indirect and direct UHT processing, Krasaekoopt *et al.* (2003) on yogurt from UHT milk, IDF (2000) and Scheldeman *et al.* (2006) on *B. sporothermodurans* and other heat-resistant sporeformers, and Al-Attabi *et al.* (2008) on volatile sulphur compounds in UHT milk. Some IDF monographs which focus on heat treatments but are not wholly devoted to UHT processing also contain much information pertinent to UHT processing, e.g. IDF (1989, 1992, 1995, 1996a). One particularly valuable source of information and advice is the manufacturers of UHT processing and aseptic packaging equipment. Some of these manufacturers, particularly APV and Tetra Pak, publish booklets that are informative, well illustrated and easy to read. A practical manual for quality assessment of UHT milks is another useful publication (Newstead, 2000).

13.8   References

ADAMS D M, BARACH J T and SPECK M L (1975), 'Heat-resistant proteases produced in raw milk by psychrotrophic bacteria of dairy origin', *J Dairy Sci*, 58, 828–834.

AHN J, BALASUBRANANIUM V M and YOUSEF A E (2007), 'Inactivation kinetics of selected aerobic and anerobic bacterial spores', *Int J Food Microbiol*, 113(3), 321–329.

AL-ATTABI Z, D'ARCY B R and DEETH H C (2008), 'Volatile sulphur compounds in UHT milk', *Crit Rev Food Sci Nutr*, 49, 28–47.

AL-SAADI J S and DEETH H C (2008), 'Cross-linking of proteins and other changes in UHT milk during storage at different temperatures', *Aust J Dairy Technol*, 63, 93–99.

ANON (2000), 'Zap! Better-tasting UHT milks!', *Food Engineering*, 72, 14–15.

BAKE K (1997), 'Tetra Therm Aseptic Plus Two – a new UHT line for all applications', *Deutsche Milchwirtschaft*, 48, 514–516.

BELMAR-BEINY M T, GOTHAM S M, PATERSON W R and FRYER P J (1993), 'The effect of Reynolds Number and fluid temperature in whey protein fouling', *J Food Eng*, 19(2), 119–139.

BOUMPA T, TSIOULPAS A, GRANDISON A S and LEWIS M J (2008), 'Effects of phosphates and citrates on sediment formation in UHT goats' milk', *J Dairy Res*, 75(2), 160–166.

BROWNING E, LEWIS M and MACDOUGALL D (2001), 'Predicting safety and quality parameters for UHT-processed milks', *Int J Dairy Tech*, 54, 111–120.

BURTON H (1988), *Ultra-high-temperature Processing of Milk and Milk Products*, London, Elsevier Applied Science.

CALVO M M and DE LA HOZ L (1992), 'Flavour of heated milks. A review', *Int Dairy J*, 2, 69–81.

CALVO M M, KLETT J M, SANTOS M P and OLANO A (1987), 'Comparative study of lactulose determination in milk by means of gas chromatography and liquid chromatography', *Rev Esp Lech*, 16, 11–13.

CATTANEO S, MASOTTI F and PELLEGRINO L (2008), 'Effects of overprocessing on heat damage of UHT milk', *Eur Food Res Technol*, 226, 1099–1106.

CERF O and DAVEY K R (2001), 'An explanation of non-sterile leaky milk packs in well-operated UHT plants', *Trans Inst Chem Eng C Food Bioprod Proc,* 79, 219–222.

CLARE D A, BANG W S, CARTWRIGHT G, DRAKE M A, CORONEL P and SIMUNOVIC J (2005), 'Comparison of sensory, microbiological, and biochemical parameters of microwaves versus indirect UHT fluid skim milk during storage', *J Dairy Sci*, 88, 4172–4182.

CODEX ALIMENTARIUS (2004), *Draft Code of Hygienic Practice for Milk and Milk Products at Step 8*, ALINORM 04/27/13, Appendix II.

COELHO P S, SILVA N, BRESCIA M V and SIQUEIRA A P (2001), 'Microbiological aspects of UHT whole milk commercialized in Belo Horizonte, Brazil', *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 53, 256–262.

CREAMER L K, BIENVENUE A, NILSSON H, PAULSSON M, VAN WANROIJ M, LOWE E K, ANEMA S G, BOLAND M J and NEZ-FLORES R J (2004), 'Heat-induced redistribution of disulfide bonds in milk proteins 1. Bovine β-lactoglobulin', *J Agric Food Chem*, 52, 7660–7668.

CUNHA M F (2001), 'Review: UHT milk and the age gelation phenomena', *Boletim do Centro de Pesquisa e Processamento de Alimentos*, 19, 341–352.

DANNENBERG F and KESSLER H G (1988), 'Reaction kinetics of the denaturation of whey proteins in milk', *J Food Sci*, 53, 258.

DATTA N and DEETH H C (2001), 'Age gelation of UHT milk – a review', *Trans Inst Chem Eng C: Food Bioprod Proc*, 79, 197–210.

DATTA N and DEETH H C (2003), 'Diagnosing the cause of proteolysis in UHT milk', *LWT Food Sci Technol*, 36(2), 173–182.

DATTA N and DEETH H C (2005), 'UHT and aseptic processing of milk', in Tewari G and Juneja V, *Advances in Thermal and Non-thermal Processing*, Ames, IA, Blackwell Publishing, 63–90.

DATTA N, ELLIOTT A J, PERKINS M L and DEETH H C (2002), 'Ultra-high temperature (UHT) treatment of milk: Comparison of direct and indirect modes of heating', *Aust J Dairy Technol*, 57, 211–227.

DE BLOCK J, MERCHIERS M, VAN RENTERGHEM R and MOERMANS R (1996), 'Evaluation of two methods for the determination of lactulose in milk', *Int Dairy J*, 6, 217–222.

DEETH H C (1999), 'Electrical heating: Current passage tube technology', *Aust J Dairy Technol*, 54, 66–68.

DEETH H C and DATTA N (2002), 'Alternative technologies for producing sterile low-acid food products', *Food Australia*, 54, 273–277.

DEETH H C and DATTA N (2003), 'Ultra-high temperature treatment (UHT): Heating systems', in Roginski H, Fuquay J W and Fox P F, *Encyclopedia of Dairy Sciences*, London, Academic Press, 2642–2652.

ELLIOTT A J, DATTA N, AMENU B and DEETH H C (2005), 'Heat-induced and other chemical changes in commercial UHT milks', *J Dairy Res*, 72, 442–446.

FORSCHINO R, GAILL A and OTTOGALLI G (1990), 'Research on the microflora of UHT milk', *Annali di Microbiologia ed Enzymologia*, 40, 47–59.

GAUCHER I, MOLLE D, GAGNAIRE V and GAUCHERON F (2008), 'Effects of storage temperature on physico-chemical characteristics of semi-skimmed UHT milk', *Food Hydrocolloids*, 22, 130–143.

GILMOUR A and ROWE M T (1990), 'Microorganisms associated with milk in dairy microbiology', in Robinson R K, *Dairy Microbiology*, vol 1, 2nd edition, London, Elsevier Applied Science, 37–76.

GRIJSPEERDT K, MORTIER L, DE BLOCK J and VAN RENTERGHEM R (2004), 'Applications of

modelling to optimise ultra high temperature milk heat exchangers with respect to fouling', *Food Control*, 15, 117–130.

GUPTA G, HOLLAND J W, DEETH H C and ALEWOOD P F (2007), 'Identification of deamidation in milk proteins after UHT treatment by proteomics analysis', *12th Lorne Proteomics Symposium*, February 2007, Lorne, Victoria, Australia.

HAMMER P, LEMBKE F, SUHREN, G and HEESSCHEN W (1996), 'Characterization of a heat-resistant mesophilic *Bacillus* species affecting the quality of UHT milk', in *Heat Treatments and Alternative Methods*, Document 9602, Brussels, International Dairy Federation, 9–16.

HARDHAM J F, IMISON B W and FRENCH H M (2000), 'Effect of homogenisation and micro-fluidisation on the extent of fat separation during storage of UHT milk', *Aust J Dairy Technol*, 55, 16–22.

HARYANI S, DATTA N, ELLIOTT A J and DEETH H C (2003), 'Production of proteinases by psychrotrophic bacteria in raw milk stored at low temperature', *Aust J Dairy Technol*, 58 16–20.

HASSAN A N, ZAHNRAN A S, METWALLI N H and SHALABI S I (1993), 'Aerobic spore-forming bacteria isolated from UHT milk produced in Egypt', *Egypt J Dairy Sci*, 21, 109–121.

HENLE T, SCHWARZENBOLZ U and KLOSTERMEYER H (1996), 'Irreversible crosslinking of casein during storage of UHT-treated skim milk', in *Heat Treatments and Alternative Methods*, Brussels, International Dairy Federation, 290–298.

HILLBRICK G C, MCMAHON D J and DEETH H C (1998), 'Electrical impedance particle size method (Coulter counter) detects the large fat globules in poorly homogenised UHT processed milk', *Aust J Dairy Technol*, 53, 17–21.

HINRICKS J and RADEMACHER B (2003), 'Sterilisation of milk and other products', in Roginski H, Fuquay J W and Fox P F, *Encyclopedia of Dairy Sciences*, London, Academic Press, 2569–2577.

HOSTETTLER H (1972), 'History of the development of UHT processes', in *IDF Monograph on UHT Milk*, Document 68, Brussels, International Dairy Federation, 169–174.

HUIJS G, VAN ASSELT A, VERDURMEN R and DE JONG P (2004), 'High speed milk', *Dairy Ind Int*, 6911, 30–32.

IAMETTI S and BONOMI F (1996), 'Transient modification of the association equilibrium in heated β-lactoglobulin', in *Heat Treatments and Alternative Methods*, Document 9602, Brussels, International Dairy Federation, 341–349.

IDF (1981), *New Monograph on UHT Milk*, Document 133, Brussels, International Dairy Federation.

IDF (1989), *Heat-induced Changes in Milk*, Fox P F (ed.), Document 238, Brussels, International Dairy Federation.

IDF (1992), *Protein and Fat Globule Modifications by Heat Treatment, Homogenisation and Other Technological Means for High Quality Dairy Products*, Document 9303, Brussels, International Dairy Federation.

IDF (1995), *Heat-induced Changes in Milk*, Document 9501, Brussels, International Dairy Federation.

IDF (1996a), *Heat Treatments and Alternative Methods*, Document 9602, Brussels, International Dairy Federation.

IDF (1996b), *UHT Cream*, Document 315, Brussels, International Dairy Federation, 3–34.

IDF (2000), Bacillus sporothermodurans – *a Bacillus Forming Highly Heat-resistant Spores*, Document 357, Brussels, International Dairy Federation, 1–27.

INTARAPHAN I (2001), *Heat-resistant sporeformers in UHT milk*, PhD thesis, University of Queensland, Australia.

JEURNINK T J M, WALSTRA P and DE KRUIF C G (1996), 'Mechanisms of fouling in dairy processing', *Neth Milk Dairy J*, 50, 407–426.

KALOGRIDOU-VASSILIADOU D (1992), 'Biochemical activities of *Bacillus* species isolated from flat sour evaporated milk', *J Dairy Sci*, 75, 2681–2686.

KELLY A, DATTA N and DEETH H C (2005), 'Thermal processing of dairy products', in Sun D-W, *Thermal Food Processing: New Technologies and Quality Issues*, Boca Raton, FL, Taylor & Francis, 265–298.

KESSLER H G (1981), *Food Engineering and Dairy Technology*, Freising, Germany, Verlag A. Kessler.

KESSLER H G (1994), 'Heat resistance of microorganisms under seals', *Proc 24th Int Dairy Congr, Melbourne*, Brussels, International Dairy Federation.

KIESNER C, MEISEL H and LOSCH M (2004), 'Report on the complementary test of the ultra-high heating plant of the type TA-FLEX-Turbo (Steritube, TA-FLEX)', *Kieler Milchwirtschaftliche Forschungsberichte*, 56, 203–218.

KOCAK H R and ZADOW J G (1985), 'Controlling age gelation of UHT milk with additives', *Aust J Dairy Technol*, 40(2), 58–64.

KOSARIC N, KITCHEN B, PANCHAL C J, SHEPPARD J D, KENNEDY K and SERGEANT A (1981), 'UHT milk: Production, quality and economics', *Crit Rev Food Sci Nutr*, 21, 153–199.

KRASAEKOOPT W, BHANDARI B and DEETH H C (2003), 'Yogurt from UHT milk: A review', *Aust J Dairy Technol*, 58, 26–29.

LEFEBVRE S and LEULIET J C (1997), 'Laminar mixed convection in constant wall heat flux tubular heat exchangers', in Lowitt R, *Engineering and Food at the Seventh International Congress of Engineering and Food ICEF7*, Part 1, Sheffield, Academic Press, C97–C100.

LEVITON A, ANDERSON H A, VETTEL H E and VESTEL J H (1963), 'Retardation of gelation in high-temperature short-time sterile milk concentrates with polyphosphates', *J Dairy Sci*, 46(4), 310–319.

LEWIS M and HEPPELL N (2000), *Continuous Thermal Processing of Foods: Pasteurization and UHT Sterilization*, Gaithersburg, MD, Aspen Publishers.

LÜCK H, MOSTERT J F and HUSMANN R A (1978), 'Bacteriological evaluation of UHT milk', *South Afr J Dairy Technol*, 10, 83–85.

LYSTER R L J (1970), 'The denaturation of α-lactabumin and β-lactoglobulin in heated milk', *J Dairy Res*, 37, 233–243.

MANZI P and PIZZOFERRATO L (2006), 'UHT thermal processing of milk', in Sun D-W, *Thermal Food Processing: New Technologies and Quality Issues*, Boca Raton, FL, CRC Press, 299–334.

MARSHALL R T (1996), 'Critical point to control contamination, growth and enzymatic activity', *Symposium on Bacteriological Quality of Raw Milk*, Wolfpassing, Austria, 13–15 March, Brussels, International Dairy Federation.

MCMAHON D J (1996), 'Age-gelation of UHT milk: Changes that occur during storage on shelf-life and the mechanism by which age-gelation occurs', in *Heat Treatments and Alternative Methods*, Document 9602, Brussels, International Dairy Federation, 315–325.

MEIER J, RADEMACHER B, WALENTA W and KESSLER H G (1995), 'Heat resistant spores under UHT treatment', in *Heat Treatments and Alternative Methods*, Document 9602, Brussels, International Dairy Federation, 17–25.

MESSIA M C, CANDIGLIOTA T and MARCONI E (2007), 'Assessment of quality and technological characterisation of lactose-hydrolysed milk', *Food Chem*, 104, 910–917.

MONTARON M P, DE STOUTZ J, COSSON J L and GERVAIS P (1991), 'Le traitement thermique des fluides par effect Joule', *Industries Alimentaires et Agricoles*, 108, 833–837.

MUIR D D (1984), 'UHT-sterilized milk concentrate: a review of practical methods of production', *J Soc Dairy Technol*, 37, 135–141.

MUIR D D (1990), 'The microbiology of heat-treated fluid milk products', in Robinson R K, *Dairy Microbiology, Vol 1*, New York, Elsevier Applied Science, 209–243.

MUIR D D (1996), 'The shelf life of dairy products: III. Factors influencing intermediate and long life dairy products', *J Soc Dairy Technol*, 49(1), 67–72.

NEWSTEAD D F (ed.) (2000), *Methods for Quality Assessment of UHT Milks*, 2nd edition, Palmerston North, New Zealand, New Zealand Dairy Research Institute.

NEWSTEAD D F, PATERSON G, ANEMA S G, COKER C J and WEWALA A R (2006), 'Plasmin activity in direct-steam-injection UHT-processed reconstituted milk: effects of preheat treatment', *Int Dairy J*, 16, 573–579.

PEARCE L E (2004), 'Survey of data on heat resistance of dairy pathogens', *IDF Bulletin 392*, Brussels, International Dairy Federation, 37–41.

PERKINS M L and DEETH H C (2001), 'A survey of Australian consumers' attitudes towards UHT milk', *Aust J Dairy Technol*, 56, 28–34.

PERKINS M L, ELLIOT A J, D'ARCY B R and DEETH H C (2005), 'Stale flavour volatiles in Australian commercial UHT milk during storage', *Aust J Dairy Technol*, 60, 231–237.

PERKINS M L, ZERDIN K, ROONEY M L, D'ARCY B R and DEETH H C (2007), 'Active packaging of UHT milk to prevent the development of stale flavour during storage', *Packaging Technol Sci*, 20(2), 137–146.

PETTERSSON B, LEMBKE F, HAMMER P, STACKEBRANT E and PRIEST G (1996), '*Bacillus sporothermodurans*, a new species producing highly heat-resistant endospores', *Int J Systematic Bacteriol*, 46, 759–764.

PRAKASH S (2007), *Burn-on in ultra-high-temperature processing of milk*, PhD thesis, University of Queensland, Australia.

RESMINI P and PELLEGRINO L (1994), 'HPLC of furosine for evaluating Maillard reaction damage in skim milk powders during processing and storage', *IDF Bulletin 298*, Brussels, International Dairy Federation, 31–36.

REZNIK D (1996), 'Ohmic heating of fluid foods', *Food Technol*, 50(5), 250–251.

ROBERTSON G (2002), 'The paper beverage carton: past and future', *Food Technol*, 56, 46–48, 50–52.

ROBERTSON G L (2003), 'Ultra-high temperature treatment (UHT): Aseptic packaging', in Roginski H, Fuquay J H and Fox P F, *Encyclopaedia of Dairy Sciences*, London, Academic Press, 2637–2642.

ROBERTSON G L (2005), 'Aseptic packaging of foods', in *Food Packaging: Principles and Practice*, 2nd edition, Boca Raton, FL, CRC Press, 255–270.

ROSS A I V, GRIFFITHS M W, MITTAL G S and DEETH H C (2003), 'Combining nonthermal technologies to control foodborne microorganisms', *Int J Food Microbiol*, 89(2/3), 125–138.

RYSSTAD G and KOLSTAD J (2006), 'Extended shelf life milk – advances in technology', *Int J Dairy Technol*, 59(2), 85–96.

SALA F J, BURGOS J, CONDON S and LOPEZ P (1995), 'Effect of heat and ultrasound on microorganisms and enzymes', in Gould G W, *New Methods of Food Preservation*, New York, Blackie Academic and Professional, 177–204.

SCHAMBERGER G P and LABUZA T P (2007), 'Effect of green tea flavonoids on Maillard browning in UHT milk', *LWT – Food Sci Technol*, 40, 1410–1417.

SCHELDEMAN P, GOOSSENS K, RODRIGUEZ-DIAZ M, PIL A, GORIS J, HERMAN L, DE VOS P, LOGAN N A and HEYNDRICKX M (2004), '*Paenibacillus lactis* sp nov isolated from raw and heat-treated milk', *Int J System Evolut Microbiol*, 54, 885–891.

SCHELDEMAN P, HERMAN L, FOSTE S and HEYNDRICKX M (2006), '*Bacillus sporothermodurans* and other highly heat-resistant spore formers in milk', *J Appl Microbiol*, 101, 542–555.

SIMMONDS P, MOSSEL B L, INTARAPHAN T and DEETH H C (2003), 'The heat resistance of *Bacillus* spores when adhered to stainless steel and its relationship to spore hydrophobicity', *J Food Prot*, 66, 2070–2075.

SKLADAL P, MASCINI M, SALVADORI C and ZANNONI G (1993), 'Detection of bacterial contamination in sterile UHT milk using an L-lactate biosensor', *Enzyme Microbial Technol*, 15, 508–512.

SMIT F, DE JONG P, STRAATSMA J and VERSCHUEREN M (2001), [NIZO Premia as knowledge management tool for industry], *Voedingsmiddelentechnologie*, 34, 23–26.

SRICHANTRA A, NEWSTEAD D F, MCCARTHY O J and PATERSON A H J (2006), 'Effect of preheating on fouling of a pilot scale UHT sterilizing plant by recombined, reconstituted and fresh whole milks', *Food and Bioproducts Processing*, 84, 279–285.

TETRA PAK (1996), 'Long-life milk', *Dairy Handbook*, 215–213.

TOPÇU A, NUMANOGLU E and SALDAMLI I (2006), 'Proteolysis and storage stability of UHT milk produced in Turkey', *Int Dairy J*, 16, 633–638.

TRAN H, DATTA N, LEWIS M J and DEETH H C (2008), 'Predictions of some product parameters based on the processing conditions of ultra-high-temperature milk plants', *Int Dairy J*, 18, 939–944.

VAN ASSELT A J, SWEERE A P J, ROLLEMA H S and DE JONG P (2008), 'Extreme high-temperature treatment of milk with respect to plasmin inactivation', *Int Dairy J*, 18, 531–538.

VARNAM A H and SUTHERLAND J P (2001), *Milk and Milk Products – Technology, Chemistry and Microbiology*, Gaithersburg, MD, Aspen Publishers.

VAZQUEZ-LANDAVERDE P A, TORRES J A and QIAN M C (2006), 'Quantification of trace volatile sulfur compounds in milk by solid-phase microextraction and gas chromatography-pulsed flame photometric detection', *J Dairy Sci*, 89, 2919–2927.

VON BOCKELMANN B and VON BOCKELMANN I A (1998), *Long-life Products: Heat-treated, Aseptically Packed: a Guide to Quality*, Åkarp, Sweden, Bernhard von Bockelmann, Publisher.

WADSWORTH K D and BASSETTE R (1985), 'Effect of oxygen on development of off-flavours in ultrahigh-temperature milk', *J Food Prot*, 48(6), 487–493.

WAES G (1976), 'Aerobic mesophilic spores in raw milk', *Milchwissenschaft*, 31, 521–525.

14

Modelling heat processing of dairy products

N. Hotrum, M. Fox, H. van Lieverloo, E. Smit and P. de Jong,
NIZO food research, The Netherlands and M. Schutyser,
Wageningen University and Research Centre, The Netherlands

Abstract: This chapter discusses the application of computer modelling to optimise the heat processing of milk. The chapter first reviews types of heat processing equipment used in the dairy industry. Then, the types of objectives that can be achieved using model-based process optimisation are discussed. Commonly encountered deterministic and stochastic modelling approaches are addressed, before highlighting some future trends in computer modelling. The chapter includes two case studies of the optimisation of milk pasteurisation, which serve to demonstrate the main advantages and disadvantages of the different modelling approaches.

Key words: optimisation of dairy heat processing, predictive process modelling, fault tree risk analysis, assessment of microbial inactivation.

14.1 Introduction to optimisation of heat processing of milk

14.1.1 Purpose of heat processing

Thermal processing is a key unit operation in the dairy industry. The main purpose of thermal processing is to inactivate pathogenic micro-organisms, thereby making milk safe for consumption. In addition, spoilage micro-organisms and enzymes are inactivated, which has the benefit of improving the shelf-life of the product. Moreover, heat treatment is often the first essential step in further processing of milk into a wide variety of consumer products and ingredients with specific functional properties.

14.1.2   Types of heat processing

There are several types of heat treatment commonly applied in the dairy industry (de Jong, 1996): (i) thermisation for the inactivation of psychrotrophic micro-organisms, (ii) low pasteurisation (LTST) for the inactivation of psychrotrophic and pathogenic micro-organisms, (iii) high pasteurisation (HTST) for the inactivation of all micro-organisms excluding spores, and for the inactivation of all micro-organisms including spores (iv) sterilisation and (v) ultra-high temperature treatment (UHT). The desired time and temperature of heating will influence the choice of processing equipment and sensitivity of the equipment to fouling. Further, product quality parameters such as odour, taste, colour, physical stability and shelf-life are affected by the time and temperature of heat treatment. Figure 14.1 depicts the time–temperature treatment in skimmed milk required for the achievement of product safety and quality indicators and the corresponding time–temperature ranges for LTST, HTST, UHT and sterilisation processes. Here it can be observed that all four heating regimes are sufficient to achieve 99% reduction of alkaline phosphatase activity, but requirements for long shelf-life products such as 99% reduction in plasmin activity and 12D reduction *C. botulinum* can only be achieved by sterilisation. However, more intense heating is not always desirable as it leads to a higher level of Maillard browning, indicated in this case by hydroxymethyl furfural (HMF) formation, the associated cooked flavour profile and/or other negative product aspects.

The above-mentioned heat treatments are based on continuous thermal processing, which takes advantage of the fact that the activation energy of microbial destruction is higher than that of nutrient destruction. In particular, high-temperature short-time (HTST) heat processes, such as UHT, have the

Fig. 14.1   Time–temperature graph with indications of time–temperature ranges of different heat treatments and the required time–temperature combinations for formation of 20 μmol HMF (indicator for Maillard reaction), 12D inactivation of *Clostridium botulinum*, 99% reduction of plasmin and alkaline phosphatase activity in skimmed milk.

potential to realise the same level of microbial inactivation as (retort) sterilisation while minimising loss of product quality due to Maillard browning (Fig. 14.1). Compared to batch pasteurisation, continuous heat exchangers offer rapid heat transfer from the heat source to the target fluid. Heat exchange systems can be categorised as either indirect or direct. The former uses a hot fluid medium (under pressure) as the heating medium, the latter uses steam.

Indirect heat exchangers operate on a mechanism of heat transfer involving transfer of heat from a warm fluid to a cool fluid via a surface. Two main configurations, plate and tubular heating systems, are available. Plate heat exchangers consist of stacked plates forming channels, such that product and water flow through alternate channels. Heat is transferred from the warm fluid across the surface of the plate to the cool fluid. Tubular heat exchangers consist of one or more tubes through which product can flow surrounded by an outer tube through which the heating medium flows. In this configuration the surface of the tube forms the surface for heat transfer. Plate heat exchangers have the advantage of a larger specific heat exchange area compared to tubular heat exchangers. Conversely, tubular heat exchangers have the advantage of being more robust than plate heat exchangers. Both systems are widely used in the dairy industry.

Direct heat exchangers transfer heat by mixing steam with the product under pressure. Subsequently, as the steam mixes with the much colder product, condensation leads to rapid heat transfer, which is the main advantage of direct heat exchange systems compared to indirect heat exchangers. There are two ways to mix the product with steam. With steam injection, steam is injected into a flowing product stream. Alternatively, steam infusion involves spraying the product into a vessel pressurised with steam (de Jong, 1996; de Jong *et al.*, 2001). Direct heat exchange systems tend to be more costly to install and less energy efficient compared to indirect heat exchangers. For this reason, direct heat exchange is generally restricted to situations where rapid heating is required, for example in processing products susceptible to rapid fouling or when cooked flavour and Maillard browning need to be kept to a minimum.

14.1.3   Approaches to optimisation of heat processing

Safety, quality and processing costs are the driving factors for the optimisation of heat processing. Food safety is of prime concern, meaning an improved process must meet the required safety standards defined for the product. Criteria related to each aspect of optimisation can be defined and the suggested change to the process can be tested in terms of these criteria using computer-based simulation. Table 14.1 shows examples of criteria important to the development of a model for the optimisation of pasteurisation of milk intended for different uses including cheese, fresh consumption, extended shelf-life, UHT and sterilisation. Many criteria can be defined and all are important to the product properties and processing costs. Due to the many parameters involved, computer-based modelling offers the possibility to investigate the effect of

Table 14.1 Examples of criteria to be taken into consideration for optimisation of milk heat treatment

Aspect	Criteria	Description	Relevance of criteria based on product				
			Cheese milk	Fresh milk	ESL[a] milk	UHT milk	Sterilised milk
Safety	F_0-value (min)	Heat load required for a safe product	−	−	+	++	++
	12D reduction of *C. botulinum*	Pathogenic micro-organism	−	−	+	++	++
	6D reduction of *B. cereus*	Pathogenic micro-organism	−	−	++	++	++
Quality	Decimal reduction of *P. fluorescens*	Spoilage micro-organisms	+/−	+/−	++	−	−
	99% reduction of plasmin activity	Heat-stable spoilage enzyme	−	−	+	++	++
	1% residual alkaline phosphatase activity	Heat-stable spoilage enzyme	++	++	−	−	−
	Volatile sulphur compound formation	Indicator of cooked flavour	−	−	++	+	+/−
	HMF formation	Marker for Maillard browning	−	−	+/−	++	++
	Lactulose formation	Marker for nutritional quality	−	−	+/−	++	++
Processing costs	Risk of fouling	Production run- and cleaning time	+/−	+/−	+	++	−
	Specific heat exchange area	Heat transfer time	+	+	+/−	+	−
	Heat regeneration	Energy costs	++	+	+/−	+/−	−

[a] ESL = extended shelf-life.

changes to processing conditions on a variety of product properties simultaneously, while reducing the need for experimental analysis. This offers a time- and cost-efficient method to test a wide variety of processing parameters.

14.2   Modelling: focus on process, product and costs

Predictive modelling offers an efficient and powerful tool to optimise heat processes in the food industry and thereby enable the technologist to perform multiple objective optimisation studies. For example, predictive modelling can be applied to the simultaneous optimisation of heating processes with respect to product quality and safety, process design and costs.

14.2.1   Process: modelling design aspects

The implementation of a heat process into a predictive model requires the translation of equipment specifications and layout into relevant model input parameters. Depending on the level of detail required, it may be sufficient to determine:

- Level 1 – Residence time and temperature of the product in the holding tube
- Level 2 – Product flows, section volumes, exchange areas, heat transfer coefficients, temperatures in the different sections, etc.
- Level 3 – Entire geometry and design of the different hardware sections, e.g. plate design, curvatures of pipes.

The level of detail required depends very much on the situation. In industrial practice residence time and the temperature of the holder (level 1) often form the basis for calculations on the thermal inactivation of micro-organisms to ensure food safety. However, extensive optimisation taking into account product quality, process design and cost of a heat treatment protocol is preferably based on the complete temperature–time profile of a heating process. This temperature–time profile can be constructed with the additional information from level 2. For the actual design of section details it is necessary to include information about the geometry of the system. This is required, for example, for the design of steam injectors where the impact of equipment design on residence time distribution is of crucial importance (level 3).

14.2.2   Product quality and safety aspects of modelling

In many cases, thermal inactivation of micro-organisms has been found to obey first-order kinetics. The decimal reduction time (D) is used to indicate 1-log reduction in the concentration of micro-organisms ($N/N_0 = 0.1$). The temperature dependence of microbial inactivation can be taken into account by setting the temperature (T) required for 1-log reduction of the D-value (z) compared to a reference temperature (T_{ref}). In equation form:

$$\frac{N}{N_0} = 10^{-t/D} \quad \text{with} \quad \log\left(\frac{D}{D_{\text{ref}}}\right) = -\frac{T - T_{\text{ref}}}{z} \qquad\qquad 14.1$$

For modelling purposes, the Arrhenius relationship is frequently used to describe the temperature dependence of thermal inactivation of micro-organisms. The advantage of this approach is that k can be easily calculated for any given temperature:

$$N = N_0 \, e^{-kt} \quad \text{with} \quad k = k_0 \, e^{-E_a/RT} \qquad\qquad 14.2$$

where k_0 is the pre-exponential factor and E_a is the activation energy. When fitting the Arrhenius equation to experimental inactivation data, the parameters k_0 and E_a are usually highly correlated; for this reason van Boekel (1996) proposed to adapt the Arrhenius equation to:

$$k = k_0 \, e^{-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{\bar{T}}\right)} \qquad\qquad 14.3$$

where $\bar{T}$ is the average temperature in the experimental data series, which reduces the dependency between k_0 and E_a.

To describe the impact of heat processing on food quality aspects, similar models have been applied as for thermal inactivation of micro-organisms. Depending on the actual food component, different reaction orders may be used. For heat treatment of milk, the denaturation of (whey) proteins (de Jong, 1996), the formation of sulphur-containing flavours (de Wit and Nieuwenhuijse, 2008), the destruction of enzymes, such as phosphatase (de Jong, 1996) or plasmin (Rollema and Poll, 1986) and the reduction in nutrient content, such as thiamine (Bayoumi and Reuter, 1985), can be relevant topics depending on the required product properties. Moreover, depending on the matrix composition it may be of crucial importance to include additional parameters such as pH and water activity.

14.2.3   Cost aspects

In a commercial setting, the occurrence of (bio-)fouling during heating of milk has a large impact on production costs. Fouling may give rise to reduced heat transfer efficiency and, moreover, increased risk of microbial contamination. Regular cleaning, with its associated costs for energy, cleaning chemicals, product loss, water use, waste water emissions and production downtime, is required in order to keep (bio-)fouling under control. Predictive modelling can be applied to optimise the pasteurisation process to reduce the need for cleaning and/or to optimise the cleaning procedure, leading to a reduction in production costs for the food manufacturer.

14.3   Deterministic modelling approaches

Deterministic models are mathematical models that produce a point estimate of the parameters of interest (e.g. log reduction of micro-organisms or Maillard reaction product concentration). The value of the point estimate is valid for a

specific time and temperature profile. Moreover, a deterministic modelling approach requires information regarding the reaction kinetics of the parameters of interest. With the aid of deterministic models, heating processes can be optimised to almost any level of detail. However, the most straightforward approach is to estimate the holding time and temperature of the most intense heating step. In this section, the scope of the application of deterministic modelling and its limitations to optimise heat processing is illustrated with the aid of examples from the literature related to the safety and quality of milk.

14.3.1 Applications of deterministic modelling to optimise heat processing
Multi-variable optimisation
Schutyser *et al*. (2008) demonstrated the potential of deterministic modelling to optimise the pasteurisation of cheese milk. While taking into account minimum processing requirements for the inactivation of micro-organisms (e.g. *Listeria monocytogenes* and *Pseudomonas* spp.) and enzymes (e.g. milk lipase and protease), whey protein denaturation and biofilm formation were selected as additional parameters in order to optimise pasteurisation with respect to cheese yield and risk of biofouling. This multi-variable optimisation was based on combining earlier models for each of the parameters of interest. For example, thermo-resistant streptococci (TRS), such as *Streptococcus thermophilus*, are known to form biofilms, which can be a source of post-pasteurisation microbial contamination. In cheese, TRS concentrations exceeding 10^5 cfu/mL milk lead to severe taste and structure defects. De Jong *et al*. (2002a) developed predictive correlations that include the build-up of a biofouling layer with *Streptococcus thermophilus* during the pasteurisation of milk. These predictive correlations were applied by Schutyser *et al.* (2008) in cheese milk optimisation. An optimum level of whey protein denaturation is known to result in an increased cheese yield without detrimental effects on product taste. This effect arises as denatured whey proteins attach to casein micelles, thus becoming enclosed in the curd matrix and increasing cheese yield. Kinetic data describing whey protein denaturation (Dannenberg and Kessler, 1988) were applied by Schutyser *et al.* (2008) in combination with processing configuration data (type of equipment, heat exchange area, flow rate, temperature–time profile). This generated a predicted outcome of the optimisation parameters for the current cheese pasteurisation process and for potential alternative temperature–time treatments in a factory setting. The result of implementation of modifications to the pasteurisation process was an increased cheese yield (one extra cheese per 100 cheeses) and a reduction in contamination with TRS from critical to negligible without compromising other safety and quality parameters (microbial and enzyme inactivation).

Fouling reduction
Deposition of proteins and minerals on heat exchange surfaces is a problem as it causes a decrease in the heat transfer coefficient, an increase in pressure drop and a loss of product remaining on the heated wall, and poses a risk for

contamination of the processed product with loosened deposit. The cause of this serious industrial fouling problem has been investigated thoroughly (de Jong *et al.*, 1992; Jeurnink, 1996; de Jong, 1997), leading to the development of a fouling model describing the conditions for the formation of two types of deposits: A, soft, bulky material containing 50–70% w/w protein that is formed between 75ºC and 115ºC; and B, hard granular material containing up to 80% w/w minerals that is formed at temperatures >110ºC. Moreover, the model takes into account additional parameters including dry matter content of the pasteurised liquid, calcium content, pH, air content and age of milk.

The model (de Jong, 1997) is based on the correlation between protein denaturation in milk and fouling in heat exchangers that has been confirmed by many investigators (Lalande *et al.*, 1984; Schraml and Kessler, 1996; Fryer, 1989) and demonstrates that protein/mineral fouling is a heat-induced transformation. The rate of adsorption, r_F, is described by:

$$r_F = k_{adsorption} [B^*]^{1.2} \qquad\qquad 14.4$$

where B^* is the concentration of the activated β-lactoglobulin monomer. The reaction order of 1.2 indicates that the adsorption rate represents an overall reaction involving different steps. The rate constant, $k_{adsorption}$, is described by the Arrhenius relationship [14.2], where in this case T is the absolute temperature of the heat exchange surface. The Arrhenius model parameters and resulting $k_{adsorption}$ have been determined and validated for temperatures up to 115ºC (de Jong, 1996). In this model, protein/mineral fouling is a heat-induced transformation. Therefore, the main control variables are temperature, residence time and flow rate. The level of adsorption can be predicted by the fouling model [14.4] and the influence of the control parameters on the level of fouling, and subsequent level of optimisation can be determined by computer simulation, thus reducing the need for costly production trials.

Flavour optimisation

Intense heat treatments, such as UHT and sterilisation, are known to result in milk with a flavour that is significantly different from that of fresh milk. Depending on the consumer preference, a reduction in the level of heat-induced flavours may be desirable in order to improve the perceived quality of the product. Insight into the reaction mechanisms of the volatile sulphur components, known to be responsible for the heated milk flavour, can support computer-simulated flavour optimisation. Recently, de Wit and Nieuwenhuijse (2008) applied multi-response modelling to construct a quantitative mechanistic model that describes changes in volatile sulphur components and protein-bound sulphydryl groups in skimmed milk and semi-skimmed milk subjected to heat treatments corresponding to pasteurisation and sterilisation. The reaction rates were found to be temperature dependent with reaction rate constants valid at temperatures ranging from 80 to 90ºC and 100 to 135ºC, respectively. The kinetic models elucidated by de Wit and Nieuwenhuijse (2008) had an accept-

able fit and correlation to sensory data, and therefore have a predictive value for comparison of heating regimes, which will be presented in Section 14.4.

Cleaning optimisation
A further dimension in the optimisation of heating processes with respect to product quality and cost control is the in-line application of predictive models. For example, NIZO Food Research has recent experience with the in-line optimisation of heating and cleaning of heating equipment using OPTICIP$^+$ (Allersma *et al.*, 2009). Based on production parameters including flow, temperature drop and product composition, the predictive modelling software, NIZO Premia, predicts and monitors the level of fouling in the processing equipment. This information is used to select an optimum cleaning regime at the end of the production run. Moreover, during cleaning, data regarding the level of fouling in the rinsing fluid is stored in the Premic database where it is applied to signal switching of cleaning steps, to optimise cleaning conditions and optimise cleaning results. The optimisation of cleaning procedures with the aid of OPTICIP$^+$ has the advantage of increasing production capacity, reducing the amount of waste water, cleaning chemicals and product losses, thereby offering production cost savings to the food manufacturer.

14.3.2   Limitations of deterministic modelling

The certainty of the mathematical model and the random variability in product behaviour determine the accuracy of the predicted product parameters resulting from deterministic modelling. Model validation serves to increase the certainty of the parameters describing the deterministic model. The application of micro-technology has a high potential for high throughput validation experiments covering a wide range of processing conditions and different samples. With, for example, a micro-heating system, kinetic data can be collected to feed predictive models (Purwanti *et al.*, 2009).

Deterministic models do not account for the random variability that is inherent in food systems. Example sources of variability include seasonal variability in the composition and heat stability of milk, variability in bacterial load, growth and inactivation behaviour of micro-organisms and storage temperature fluctuations in the supply chain. This type of variability is a source of error in the point estimate product parameter, independent of the certainty of the parameters governing the mathematical model describing the given product characteristic. This natural variability in the behaviour of the system can be taken into account using stochastic modelling, which is addressed in Section 14.5.

14.4   Case study: application of deterministic modelling to milk sterilisation

In this section the application of deterministic modelling to assess the impact of direct and indirect sterilisation of milk on product quality and equipment fouling

is demonstrated. The NIZO developed web-based modelling tool, Websim-MILQ (Schutyser *et al.*, 2008), as well as the Q_{sim} application of NIZO Premia was used to perform the simulation. Parameters related to safety (decimal reduction of *Geobacillus stearothermophilus*), nutritional value (thiamin, free lysine), off-flavour formation (methanethiol, hydrogen sulphide), extent of Maillard reaction (5-HMF) and equipment fouling (native β-lactoglobulin, mass of fouling deposit) for plain milk were selected as key indicators of relevant safety, quality and cost aspects. The outcome of these parameters was simulated for two heating profiles based on either direct or indirect heating (Fig. 14.2). The results of the simulation (Table 14.2) demonstrate that for an identical heat load (F_0 value of 12 min) a higher level of native protein, thiamin concentration and free lysine is obtained with the direct heating method compared to the indirect heating method. In addition, the level of volatile components associated with cooked flavour and the extent of the Maillard reaction are reduced by direct

Fig. 14.2   Temperature profiles used for UHT via (a) direct and (b) indirect heating methods with the same F_0 values.

Table 14.2   Simulated impact of direct and indirect sterilisation on product quality and fouling

	Direct steam injection	Indirect heating
Processing parameter		
Holding time (s)	6.4	51.9
Holding temperature (ºC)	141.0	132.3
F_0 (min)	12	12
Component		
Geobacillus stearothermophilus[a] (decimal reduction)	6.4	6.3
Thiamin[b] (%)	95	77
Free lysine content[c] (%)	94	74
Methanethiol[d] (%, normalised to indirect heating)	20	100
Hydrogen sulphide[d] (%, normalised to indirect heating)	27	100
5-Hydroxymethylfurfural[e] (μmol/L)	7.6	24.6
Native β-lactoglobulin[f] (%)	20	1
Deposit due to fouling[g] after 12 h run time (kg), applying a product flow of 125 L/h	0.08	0.27

[a] Inactivation kinetics applied from Peri *et al.* (1985).
[b] Destruction kinetics applied from Kessler and Fink (1986).
[c] Destruction kinetics applied from Bayoumi and Reuter (1985).
[d] Formation kinetics applied from de Wit and Nieuwenhuijse (2008).
[e] Formation kinetics applied from de Jong (1996).
[f] Denaturation kinetics applied from Dannenberg and Kessler (1988).
[g] Fouling according to de Jong *et al.* (2002b).

heating. Moreover, the level of fouling is reduced, which opens the opportunity for increasing run time and/or decreasing cleaning effort. However, in this case study it can be observed that the inactivation of *Geobacillus stearothermophilus* is slightly less with the direct heating method. This serves as a reminder that care should be taken to include sufficient safety-related parameters (e.g. more than one micro-organism) in the design criteria for optimisation of thermal treatments. This case study demonstrates that a deterministic model that combines specific reaction kinetics with a process configuration is suitable for the prediction of the effect of individual conditions on product safety, quality and ease of processing, making this type of modelling a valuable tool in the optimisation of heat exchangers.

14.5   Stochastic modelling approaches

More extensive information can be obtained from, and more complex research questions can be dealt with, the application of stochastic models including prediction of the quality of products and complex food safety risk assessments (Cassin *et al.*, 1998). Risk assessment can involve specific parts of a food

processing line (Vissers *et al.*, 2006) or whole farm-to-fork models (Ebel *et al.*, 2004). A stochastic model can be subdivided into many separate phases, describing primary production, processing, transportation, storage, contamination at different stages and dose–response relations that describe infection risk. Each of these phases can contain different inactivation models, growth models or other models. The overall goal of stochastic risk assessments is to determine critical points in the food chain, which can be used by quality managers to identify areas that require attention.

In a risk model, a stochastic approach needs to be applied, such that variability of different process parameters (e.g. time, temperature), product properties (e.g. salt content, pH, water activity) and microbial properties (e.g. species, inactivation rate, dependency of inactivation rate on product properties) can be taken into account. Usually, Monte Carlo simulations are applied to run these models. In a Monte Carlo simulation, first the input parameters which will be varied are determined. For these parameters, suitable probability distribution functions (PDF) need to be defined, which describe the variability of the specific variable. Ideally, these PDFs are derived from experimental data, but in the event of a lack of data, expert estimations may be applied. In the second step, values for the input variables are drawn randomly and exhaustively (e.g. >10,000 times) from these PDFs and entered in the model and the resulting output (e.g. the number of micro-organisms in the product) is logged. The final result is a probability function describing the likelihood and extent of the outcome of interest. For example, a probability mass function (PMF) can describe the probability of discrete numbers of micro-organisms per aliquot of food, while for dose–response modelling to assess the infection risk of pathogens to consumers, the result is a PDF of infection.

14.6   Case study: application of stochastic modelling to milk pasteurisation

Stochastic modelling of milk pasteurisation can focus on the effect of variability of different parameters including holding time, holding temperature, initial concentration of micro-organisms and/or heat sensitivity of strains of micro-organisms. In this case study, the effect of varying holding temperature and heat sensitivity of microbial inactivation will be evaluated.

First, the inactivation effect of heating is shown without variability of any of the processing parameters. The pasteurisation equipment dimensions and process conditions (Table 14.3) chosen for this example were entered in NIZO Premia (de Jong *et al.*, 2002b). This software platform integrates equipment dimensions and process conditions with reaction kinetics. The resulting time–temperature profile (Fig. 14.3) shows different regimes:

- Regime 1: heating in the regenerative and heating section to 70°C
- Regime 2: holding at 70°C for 20.1 seconds
- Regime 3: cooling in the regenerative and cooling section to 5°C.

Table 14.3   Process parameters and dimensions of the pasteurisation equipment

Flow:	50,000 kg/h
Plate length:	1.324 m
Plate width:	0.46 m
Plate distance:	4 mm
Heat transfer coefficient:	2000 W/m^2/K
Product:	Whole milk
Product inlet temperature:	5°C

Section	Description	Dimension	Outlet temperature (°C)
1	Regenerative up	10 serial sections of 15 parallel plate passes	56
2	Heater	15 parallel plate passes	Varied
3	Holder	Pipe: $L = 34$ m, $D = 0.102$ m	Varied
4	Regenerative down	10 serial sections of 15 parallel plate passes	13
5	Cooler	18 plate passes	5

The time–temperature profile is used in the inactivation kinetics model of a selected (imaginary) micro-organism. In this example, first-order inactivation (see Section 14.2.2) is assumed with a mean $\ln(k_0)$ equal to 91.93 and a mean E_a equal to 2.65×10^5 J/mol.

Fig. 14.3   Example of the temperature profile and cumulative logarithmic microbial inactivation for a selected (imaginary) micro-organism during heating, holding and cooling of milk in a pasteurisation unit.

Fig. 14.4   Effect of increasing the holding temperature on the cumulative logarithmic inactivation during pasteurisation.

The result (Fig. 14.3) shows that inactivation occurs mainly in the holding section (regime 2), whereas more heat-sensitive micro-organisms may already be inactivated to a great extent in the heating section (regime 1). To limit negative effects of heating (e.g. Maillard products affecting taste and colour), it is important to take into account the entire time–temperature profile, thus preventing underestimation of inactivation or other heat treatment effects. Combination of different inactivation kinetics can result in optimal process settings. The inactivation at 70.0°C in this case is a 0.82 log reduction. In the event that higher inactivation rates are required, the profile can be recalculated for different holding temperatures (Fig. 14.4), showing that the inactivation rate rapidly increases with the temperature above 75°C. For example, an increase from 0.82 log to 4 log reduction would require a temperature increase from 70.0 to 76.0°C.

14.6.1   Variability in microbial inactivation temperature and processing temperature

Palisade @RISK can be used for generating the variation of the input variables by sampling randomly from the chosen probability distribution functions of every variable (Monte Carlo analysis). NIZO Premia can then be applied to calculate inactivation by processing the list of different combinations (iterations) of temperatures, heating, holding and cooling times as well as the heat sensitivities of the micro-organism. Further, @RISK can be used to analyse the output variability (e.g. fitting the probability distribution).

The variation observed in practice can be derived from the temperature logger of the pasteurisation equipment. Not many (if any) pasteurisers are equipped with a flow logger. This results in uncertainty about the actual flow and holding time, especially when changing from one process formulation to another and at the beginning and end of production runs.

The variability of the inactivation kinetics (heat-sensitivity) among micro-organisms is one of the largest sources of uncertainty in stochastic heat inactivation modelling. In 2006, a meta-analysis of literature on heat inactivation kinetics of micro-organisms was published by van Asselt and Zwietering (2006). The mean and standard deviation of $\log(D)$ of an organism at the reference temperature in this case study was used to estimate the variability of the inactivation kinetics. Not all variability in $\log(D)$ presented in this case study exhibits normal distribution; however, the added uncertainty of assuming a normal distribution is outweighed by the uncertainty in the data resulting from different labs, measurement methods and years. The z-value in this case study is fixed, assuming an equal response to temperature changes for all strains, which may result in under-estimation of the actual sensitivity of the microbial strain to temperature.

Due to the large variability of kinetics of strains of microbial species, it may be necessary to truncate the PDF of $\log(D)$, as estimated kinetics may deviate considerably from measured kinetics, especially when simulating with a large number of iterations (>100,000). For example, very low sensitivities may result from a non-truncated PDF leading to unrealistic values compared to values measured in practice.

In this case study, a normal distribution was chosen for $\ln(k_0)$ with an average of 91.93 and a standard error of 0.92. The distribution was truncated at both sides with a 99.9% confidence interval (Fig. 14.5). The temperature was varied by applying a lognormal distribution with an average of 0.2, a standard deviation of 0.2, truncated at 0 and 1.5°C and subsequently shifted from 0.2 to 75.8°C.

Fig. 14.5   Probability density function (area) and cumulative density function (line) of the chosen variability of the $\ln(k_0)$ of the heat inactivation kinetics of the imaginary organism.

Fig. 14.6   Variability of log inactivation during pasteurisation as a result of the variability of heat sensitivity of the micro-organism.

The result of a Monte Carlo simulation with the above-mentioned variation shows that the majority of the micro-organisms are inactivated at a holding section temperature of 76°C. However, in 5.6% of the cases, the inactivation results in less than the targeted log 4 reduction (Fig. 14.6). This demonstrates the value of the stochastic approach, simulating the variability that could occur in practice.

14.7   Future trends

Computer modelling will play an increasingly prominent role in the design and operation of food production. The following trends are foreseen.

14.7.1   Real-time 3D computational fluid dynamics (CFD)
CFD has been applied for several decades to design complex processing equipment, for example spray dryers (Straatsma *et al.*, 1999). However, simulations are still time-consuming (requiring several days). By acquiring more powerful computers and more highly developed CFD tools, the availability of real-time 3D-CFD, which can be additionally coupled to models that predict product properties, is anticipated.

14.7.2   Fault tree analyses (FTA)
FTA has potential to incorporate quantitative risk assessment regarding product safety and production costs in the design phase of food processing. For example, if undetected (and therefore unchecked) failure of the pasteurisation process

should occur, an unnoticed dairy product contamination may be the result. Other industries (e.g. aircraft, nuclear, petrochemical) that accept extremely low probabilities of highly unacceptable events (explosions, contamination) use Fault Tree Analysis (FTA) to estimate probabilities of both primary as well as secondary safety systems failing concurrently. FTA consists of Boolean Monte Carlo Analysis, where known probabilities of base events (failing valves, thermometers, computers, etc.) are used to calculate the probability of the top event (Andrews and Moss, 2002). This concept has been tested successfully for estimating probabilities of the contamination of pasteurised milk due to failure of pasteurisation equipment. In another example, FTA could focus on low temperature, low heating time and cross-contamination between raw and pasteurised milk (van Lieverloo *et al.*, 2009).

14.7.3   Predicting trained panel scores

Through the development of a sound model capable of predicting functional properties of a food product (e.g. shelf-life, flavour concentration, viscosity) it is possible to predict the scores of a trained sensory panel. This can be achieved by coupling the predicted functional properties and the panel scores via sophisticated black box models, such as neural networks (Verschueren *et al.*, 2002).

14.7.4   Model-based process control

Predictive models can be used to determine the optimal set-points during food production (de Jong *et al.*, 2002c). Based on a list of product specifications the model continuously calculates the optimal process conditions with respect to production costs, incorporating the actual amount of fouling and variation in conditions (e.g. fluctuating temperatures).

14.8   Sources of further information and advice

A critical aspect in predictive modelling is the availability of reliable kinetic data. There are different sources of microbial kinetic data, e.g. free software ComBase (http://www.combase.cc/) or commercial software Sym'Previus (http://www.symprevius.net). NIZO Food Research developed a modelling approach in which both process models and product models are included (NIZO Premia/WebSim-MILQ, http://www.milq.org). The advantage is that with the latter software an actual optimisation of a heating process, including different types of product conversions, can be carried out.

With regard to stochastic modelling of the presence and behaviour of micro-organisms in food, examples of further reading material include McKellar and Liu (2004) and Brul *et al.* (2007). A general background and manual for stochastic modelling is provided by Vose (2008).

14.9   References

ALLERSMA, D., SMIT, F., DE JONG, P. (2009), 'Reinigen op maat', *Voedingsmiddelen-technologie*, 5, 28–29.

ANDREWS, J.D., MOSS, T.R. (2002), *Reliability and Risk Assessment*, 2nd edn, Professional Engineering Publishing, London and Bury St Edmunds, UK.

BAYOUMI, E.S., REUTER, H. (1985), 'Thiamin losses during UHT treatment of whole milk', *Milchwissenschaft*, 40, 713–716.

BRUL, S., VAN GERWEN, S., ZWIETERING, M. (EDS) (2007), *Modelling Microorganisms in Food*, CRC Press, Boca Raton, FL.

CASSIN, M.H., PAOLI, G.M., LAMMERDING, A.M. (1998), 'Simulation modelling for microbial risk assessment', *Journal of Food Protection*, 61, 1560–1566.

DANNENBERG, F., KESSLER, H.G. (1988), 'Reaction kinetics of the denaturation of whey proteins in milk', *Journal of Food Science*, 53, 258–263.

DE JONG, P. (1996), *Modelling and optimization of thermal treatments in the dairy industry*, Thesis, Technical University Delft, the Netherlands.

DE JONG, P. (1997), 'Impact and control of fouling in milk processing', *Trends in Food Science and Technology*, 8, 401–405.

DE JONG, P., BOUMAN, S., VAN DER LINDEN, H.J.L.J. (1992), 'Fouling of heat treatment equipment in relation to the denaturation of β-lactoglobulin', *Journal of the Society of Dairy Technology*, 45, 3–8.

DE JONG, P., WAALEWIJ, R., VAN DER LINDEN, H.J.L.J. (1993), 'Validity of a kinetic fouling model for heat treatment of whole milk', *Lait*, 73, 293–302.

DE JONG, P., VERDURMEN, R.E.M., STEENBERGEN, A.S. (2001), 'Fun/ISI heater', International Patent WO 01/56394 A1.

DE JONG, P., TE GIFFEL, M.C., KIEZENBRINK M.C. (2002a), 'Prediction of the adherence, growth and release of microorganisms in production chains', *International Journal of Food Microbiology*, 74, 13–25.

DE JONG, P., TE GIFFEL, M.C., STRAATSMA, J., VISSERS, M.M.M. (2002b), 'Reduction of fouling and contamination by predictive models', *International Dairy Journal*, 12, 285–292.

DE JONG, P., SMIT, F., VISSERS, M.M.M., STRAATSMA, J., VERSCHUEREN, M., VAN DE WIEL, J. (2002c), 'A new process control system based on predictive kinetic models for food quality and operating costs', in: *Proceedings of the 2nd International Conference on Simulation in Food and Bio Industries* (ed. B. O'Connor, D. Thiel), SCS Europe, pp. 45–49.

DE WIT, R., NIEUWENHUIJSE, H. (2008), 'Kinetic modelling of the formation of sulphur-containing flavour components during heat-treatment of milk', *International Dairy Journal*, 18, 539–547, doi:10.1016/j.idairyj.2007.11.011.

EBEL, E., SCHLOSSER, W., KAUSE, J., ORLOSKI, K., ROBBERTS, T., NARROD, C., MALCOLM, S., COLEMAN, M., POWELL, M. (2004), 'Draft risk assessment of the public health impact of *Escherichia coli* O157:H7 in ground beef', *Journal of Food Protection*, 9, 1991–1999.

FRYER, P.J. (1989), 'The uses of fouling models in the design of food process plants', *Journal of the Society of Dairy Technology*, 45, 23–29.

JEURNINK, T.J.M. (1996), *Milk fouling in heat exchangers*, Thesis, Wageningen University, the Netherlands.

KESSLER, H.G., FINK, R. (1986), 'Changes in heated and stored milk with an interpretation by reaction kinetics', *Journal of Food Science*, 51, 1105–1111.

LALANDE, M., TISSIER, J.P., CORRIEU, G. (1984), 'Fouling of heat transfer surfaces related to β-lactoglobulin denaturation during heat processing of milk', *Biotechnology Progress*, 1, 131–139.

MCKELLAR, R.C., LIU, X. (EDS) (2004), *Modelling Microbial Responses in Food*, CRC Press, Boca Raton, FL.

PERI, C., PAGLIARINI, E., PIERUCCI, S. (1985), 'A study on optimizing heat treatment of milk. I. Pasteurisation', *Milchwissenschaft*, 43, 636–639.

PURWANTI, N., FOX, M.B., SCHROËN, K., DE JONG, P. (2009), 'The micro heater: a new tool for rapid determination of food kinetics', *Journal of Food Engineering*, 91, 78–84.

ROLLEMA, H.S., POLL, J.J. (1986), 'The alkaline milk proteinase system: kinetics and mechanism of heat inactivation', *Milchwissenschaft*, 41, 536–540.

SCHRAML, J.E., KESSLER, H.G. (1996), 'Effects of concentration on fouling of whey', *Milchwissenschaft*, 51, 151–154.

SCHUTYSER M., STRAATSMA J., KEIJZER P., DE JONG, P. (2008), 'A new web-based modelling tool (Websim-MILQ) aimed at optimisation of thermal treatments in the dairy industry', *International Journal of Food Microbiology*, 128, 153–157, doi:10.1016/j.ijfoodmicro.2008.07.003.

STRAATSMA, J., VAN HOUWELINGEN, G., STEENBERGEN, A.E., DE JONG, P. (1999), 'Spray drying of food products: 1. Simulation model', *Journal of Food Engineering*, 42, 67–72.

VAN ASSELT, E.D., ZWIETERING, M.H. (2006), 'A systematic approach to determine global thermal inactivation parameters for various food pathogens', *International Journal of Food Microbiology*, 107, 73–82.

VAN BOEKEL, M.A.J.S. (1996), 'Statistical aspects of kinetic modeling for food science problems', *Journal of Food Science*, 61, 477–485.

VAN LIEVERLOO, J.H.M., WILLIG, C.P., FOX, M.B., WELLS-BENNIK, M.J.H. (2009), 'Estimating probability of undetected failure of pasteurization process control using Fault Tree Analysis', *6th International Conference on Predictive Modeling in Foods*, 8–12 September 2009, Washington, DC.

VERSCHUEREN, M., VAN DEN BERG, G., DE JONG, P. (2002), 'Predicting taste development of cheese using hybrid modelling'. *Australian Journal of Dairy Technology*, 57(2), 165.

VISSERS, M.M.M., DRIEHUIS, F., TE GIFFEL, M.C., DE JONG, P., LANKVELD, J.M.G. (2006), 'Improving farm management by modelling the contamination of farm tank milk with butyric acid bacteria', *Journal of Dairy Science*, 89, 850–858.

VOSE, D. (2008), *Risk Analysis. A Quantitative Guide*, 3rd edn, John Wiley & Sons, Chichester, UK.

15

Removal of bacteria, spores and somatic cells from milk by centrifugation and microfiltration techniques

G. Gésan-Guiziou, INRA-Agrocampus Ouest, France

Abstract: Bactofugation (or centrifugation) and microfiltration are commercial and effective techniques for the physical removal of bacteria, spores and somatic cells from milk. Centrifugation is applied to separate components (microorganisms, fat globules, insoluble proteins, etc.) that have different densities compared to the dispersing phase, and microfiltration is mainly based on particle size exclusion using a semi-permeable membrane. This chapter gives a description of these two technologies (principle and operating conditions). It presents the performances of both techniques in particular with respect to the removal of bacteria, spores and somatic cells. Finally it discusses their possibilities and limitations in the dairy industry.

Key words: bacteria removal, milk, centrifugation, bactofugation, microfiltration.

15.1   Introduction

The bacteriological quality of milk is the most variable of the factors with which the cheese-maker has to contend. A wide variety of microorganisms can lead to various defects in cheese. For instance, *Clostridium* spores, especially *Cl. tyrobutyricum*, coliforms and yeasts can cause gas formation, and *Bacillus* spores and *Pseudomonas* species can produce proteolytic and lipolytic enzymes affecting mainly hard and semi-hard cheese quality. Pathogenic bacteria, such as *Staphylococcus aureus*, can also contaminate milk.

Moreover, milks with high levels of somatic cells are known to adversely affect cheese making (coagulation time, yield, and quality of cheese, especially flavour and texture) (te Giffel and van der Horst, 2004). They have been shown to have a protecting effect on *Listeria monocytogenes* during heat treatment (Doyle *et al*., 1987; Griffiths, 1989). The presence of enzymes (catalases, proteases and lipases) in somatic cells has also putative effects on cheese quality and yield (Maubois, 2002).

Since it is not possible to heat milk to such a degree that bacteria and spores can be eliminated without affecting the flavour, the functionalities of the milk components and then the cheese-making properties, non-thermal preservation technologies can be seen as an important processing stage in today's dairy plants. The non-thermal preservation technologies, such as ultra-high pressure, pulsed electric fields, ozonation and carbon dioxide technologies, are therefore the subject of intense research efforts particularly in research laboratories around the world. Many of these technologies have already found commercial application in small niches, but further development is needed in each of them to make them widely applicable.

Among them, microfiltration and bactofugation (or centrifugation) are based on the physical removal of somatic cells, bacteria and spores. These processes can be differentiated according to the physical properties which they utilize and the forces arising from them. Centrifugation is applied to separate components (microorganisms, fat globules, insoluble proteins, etc.) that have different densities compared to the dispersing phase, and microfiltration is mainly based on particle size exclusion using a semi-permeable membrane. This chapter gives a description of these two technologies (principles and operating conditions). It presents the performances of both techniques in particular with respect to the removal of bacteria, spores and somatic cells. Finally it discusses their possibilities and limitations in the dairy industry.

15.2 Centrifugation

Separation by centrifugation is based on differences in density between particles and the dispersing phase (plasma). Centrifugation is usually applied to separate fat globules in the form of cream. As milk fat has a lower density than plasma, the fat globules rise under the influence of gravity, and the rate of rising is increased when a centrifugal field is applied. Centrifugation is also used to separate particles that have a density larger than that of milk plasma. It concerns dirt particles, somatic cells and even microorganisms (bacteria, spores).

15.2.1 Stokes' law and centrifuge acceleration
As a result of the combined action of buoyancy and friction, the sedimentation speed, v ($\mathrm{m\,s^{-1}}$), of a particle of diameter d (m) in a liquid phase can be expressed according to Stokes' law:

$$v = \frac{d^2}{18\eta} \left(\rho_p - \rho_f\right) g \qquad\qquad 15.1$$

where η is the dynamic viscosity of the liquid phase ($kg\,m^{-1}\,s^{-1}$ or Pa s), ρ_p and ρ_f are the densities ($kg\,m^{-3}$) of the particle and of the dispersing phase, respectively, and g is the acceleration due to gravity ($9.81\,m\,s^{-2}$). If ρ_f is greater than ρ_p, v is negative: the particle is less dense than the liquid phase; it rises at the surface of the liquid as observed in milk creaming. If ρ_f is lower than ρ_p, the particle settles as observed in clarification processes.

Stokes' law must be used with care and several conditions must be fulfilled (Mulder and Walstra, 1974), but the equation is quite useful to predict trends. As the equation shows, particle size determines the separation efficiency. When the fat globules are present partly in floccules or clusters, as happens in raw milk (agglutination), they rise much faster than if they were separate globules because of their larger size. For a given particle size distribution, the sedimentation speed, v, depends on the difference in density between the particles and the liquid phase and on the viscosity of the liquid. In a general manner, the difference in density does not sharply vary with the temperature. However, the temperature is known to significantly affect the viscosity and then plays a major role in the separation efficiency. This is the reason why the milk fat separation is classically performed at a temperature ranging from 50°C to 60°C (viscosity close to $0.9 \times 10^{-3}\,Pa\,s$) compared to 20°C ($2.0 \times 10^{-3}\,Pa\,s$). One can note, however, that somatic cells and several bacteria participate in the cold agglutination of fat globules in raw or thermized milk (Walstra *et al.*, 2006). No agglutination occurs at 37°C, and the further the temperature falls below 35°C, the more cells are removed with the cream.

The increase of temperature is the major parameter able to increase the rate of removal, due to increase in viscosity. However, some limitations, especially due to protein denaturation, appear at temperatures higher than 60–65°C. In order to significantly increase the separation efficiency, the acceleration due to gravity can be replaced by centrifugal acceleration. The separation is then performed under a centrifugal field. In that context, Stokes' law still holds with the centrifugal acceleration defined as $R\omega^2$, where R is the effective radius of the centrifuge (m), and ω the angular velocity ($rad\,s^{-1}$), which equals $2\pi n/60$ (n is the number of revolutions per minute). The sedimentation speed is then defined as:

$$v = \frac{d^2}{18\eta} \left(\rho_p - \rho_f\right) R\omega^2 \qquad\qquad 15.2$$

As compared to natural decantation, centrifugal separation is far quicker and far more complete. The high separation performances are achieved by:

- adopting a flow-through process
- increasing the speed of the particle by means of a centrifugal acceleration
- limiting the distance over which the particles have to move, by dividing the space in which separation occurs into thin compartments with conical discs.

15.2.2   Principle of a bactofuge

Centrifugation is usually applied to separate fat globules. Clarification using a centrifuge is rarely used in the dairy industry except for the removal of bacteria/ spores from products (mainly milk) that are minimally pasteurized. Despite the quite small size of spores (for the most part 1 to 1.5 μm), this clarification is applicable, especially for spore removal, as the density difference between milk (1.028–1.038 g mL^{-1}) and bacterial spores (1.30–1.32 g mL^{-1}) is enhanced due to the concentrated cell plasma of the spores. Normal vegetative bacteria have a much lower density (1.07–1.12 g mL^{-1}) and are more difficult to remove. This centrifugation is often referred to as bactofugation because the commercial equipment manufactured by Tetra Pak is marketed under the trademark of Bactofuge^{TM}. However, other manufacturers produce centrifuges for this purpose.

In order to remove bacteria, spores and somatic cells, some specific centrifuges, also called bactofuges, have been designed. The first bactofuge was developed a few decades ago to separate mechanically the microorganisms from the milk to prevent late blowing of semi-hard cheeses by *Clostridium tyrobutyricum* (Waes and van Heddeghem, 1990). At present the bactofuges available on the market, mainly manufactured by GEA Westfalia and Tetra Pak/ Alfa-Laval, are the third generation of centrifuge separators (Waes and van Heddeghem, 1990; te Giffel and van der Horst, 2004). This generation of bactofuge is hermetic and self-desludging, whereby the concentrate is removed under pressure.

Two main categories of bactofuges are currently used. The first type is a one-phase type bactofuge and is like a normal clarifier. It has only one outlet at the top of the bactofuge for the bacteria-reduced milk, whilst the portion containing particles (bactofugate) is collected as a sludge in the bowl and discharged continuously or by intervals through a part in the bowl body. In some equipment, a continuous centrifugate stream (about 3% of the milk feed) can be recycled through the centrifuge and a discontinuous bacteria-rich portion, making up around 0.2% of the milk, is ejected periodically (every 15–20 minutes). The second type is a two-phase type bactofuge and is more like a cream separator. It has two outlets at the top, i.e. one for the bacteria-reduced milk and one for the continuous discharge of the bactofugate (about 3% of the total liquid flow) via a special top disc. In such a bactofuge, the milk to be treated enters the machine along the central axis. It enters a stack of conical discs and the flow is divided over the numerous slits between discs. The centrifugal force drives the bacteria/ spores in each slit towards the periphery of the bowl, and the bacteria-reduced milk moves towards the central axis of the centrifuge. Both streams then move up and remain separated, before being discharged from the bactofuge.

Classically, a centrifugal force of about 9000g is used in this process. It is a fast process, typically taking less than 1 s for passage of the milk through the centrifuge. The separation is performed at 55–60ºC. In respect of clarification efficiency of non-milk solids, temperature has little influence and the process can be performed at a cold or a warm temperature (3–12ºC or 52–58ºC).

However, if bacteria and spores are to be removed, only warm-milk clarification is efficient. The temperature of bactofugation of the milk is similar to that used in the cream separator, and the bactofuge is normally installed in series with the centrifugal separator, as the latter machine is used to standardize the fat content in the cheese milk.

15.2.3   Bactofugation performance and gains in milk processing

The efficiency of the milk bactofugation has been studied by various authors using first, second and third generations of centrifuges. As suggested by Waes and van Heddeghem (1990), several factors influence the efficiency of bacto-fugation. Some are related to the microorganisms' characteristics:

- Size (0.5–7.0 µm), form (spherical or rod-shaped) and characteristics of the outer surface (rough or smooth) of the microorganisms
- Density of the microorganisms
- Ability to agglomerate between bacteria themselves or between bacteria and milk ingredients
- Bacteriological quality of the milk, which may lead to chemical or physical changes.

Others are related to process parameters and constructional details of the centrifuge:

- Temperature of bactofugation (50–68ºC), which plays a major role in viscosity
- Capacity of the machine, e.g. the rate of milk flow
- Centrifugal force, e.g. the speed of revolution of the machine
- Space between the disc stack
- Design of the bactofuge, which should avoid recontamination of the centrifuged milk.

It then becomes difficult to compare performances obtained in various studies owing to the different operating conditions.

It can, however, be concluded that when operating in the temperature range of 55–60ºC, recent machines generally lead to 86.0–92.0% reduction of the total bacterial count, which corresponds to a decimal reduction of about 1 log. Compared to bacteria, the process is more effective for the removal of spores. One can generally consider that the efficiency of spore removal is substantial and ranges from 90 to 98% (decimal reduction ranging from 1.0 up to 1.7): bactofugation removes 97.4–98.7% of anaerobic spores such as *Clostridium* (see Table 15.1) and 94.1–97.7% of aerobic spores such as *Bacillus* (see Table 15.2).

Not much has been reported about the use of bactofugation for removal of somatic cells. However, it has been clearly determined that the best removal rate of somatic cells can be achieved with bactofugation, and not with classical centrifugation used to separate raw milk and cream (see Table 15.2): 95% of somatic cells can be removed by bactofugation but only 30–75% by classical centrifugation.

Table 15.1   Removal of anaerobic spores from milk by third-generation centrifuges

Type (nominal capacity)	Rate of flow (L h^{-1})	Temperature (°C)	Efficiency towards anaerobic spores (%)
GEA Westfalia	15,000	62–65	86.2
CNB 130	15,000	60	97.4
(10,000–15,000 L h^{-1})	10,000	50	98.7
Alfa-Laval BMRPX 618 GV (25,000 L h^{-1})	25,000	68	98.1
GEA Westfalia CNB 215 (25,000 L h^{-1})	25,000	62	97.8

Source: adapted from Waes and van Heddighem (1990).

Simultaneously, during bactofugation, there is a loss of milk components, in particular protein. The bacteria-rich portion has an enhanced content of casein micelles. The protein loss is determined by the type of centrifuge and the amounts of concentrate removed. In recent machines, the quantity of the concentrate can be limited to 2.5–3.5% of the total milk, with a protein level of between 2.5 and 12.8%.

The bactofugation of milk was originally developed in the cheese industry, and its most important application has proved to be the removal from milk for cheese manufacture of bacterial spores of organisms that undergo a late acid

Table 15.2   Average bacteria removal by bactofugation and centrifugation

	Bactofugation		Centrifugation (classical cream separator)		References
	Efficiency (%)	Decimal reduction (–)	Efficiency (%)	Decimal reduction (–)	
Total count	86.0–92.0[a]	0.85–1.10			van den Berg et al. (1986)
Anaerobic spores	97.4–98.7[b]	1.58–1.88			van den Berg et al. (1986)
Aerobic spores	94.1–97.7[b]	1.23–1.64			van den Berg et al. (1986)
Somatic cells	95	1.30	30–50	0.15–0.30	Wieking (2004)
			75	0.6	Saboya and Maubois (2000); Maubois (2002)

[a] Bactofugation temperature 55–65°C.
[b] Bactofugation temperature 48°C.

fermentation ('late blowing' defect). Bactofugation is not widely used in general cheese making, but has been commonly used since the 1960s for the removal of butyric acid bacterial spores, e.g. *Clostridium tyrobutyricum* and *Cl. butyricum* from milk for Dutch and Swiss-type cheeses. Since spores can lead to significant quality defects in hard cheese, semi-hard cheeses or long-life products due to proteolysis, lipolysis and gas formation, bactofugation is used mainly in the manufacture of these product groups.

The question of whether or not bactofugation of cheese-milk is effective in preventing acid fermentation in cheese has been dealt with by a number of authors (Waes and van Heddeghem, 1990). In some cases, the acid fermentation is inhibited when cheeses are prepared from bactofugated milk (Grana and Jarlsberg cheeses). The use of these separators and the addition of the sterilized bacterial concentrate to the cheese milk allowed a cheese-making procedure without any alterations (van den Berg *et al*., 1986). In some other cases, for example semi-hard, Gouda-type cheeses, the spore-reducing effect obtained by one-step bactofugation is not sufficient, especially in winter, where the content of spores is high due to silage feeding of the cows. To reduce effectively the load of bacterial spores, a double bactofugation is practised in the cheese industry (spore removal up to 99%). A decrease of the flow rate of milk, which leads to an increase in the residence time of milk in the centrifuge, can also improve the efficiency of spore removal. In most cases, however, a certain amount of nitrate still has to be added to suppress growth of butyric acid bacteria (van den Berg *et al*., 1986; Waes and van Heddeghem, 1990; *International Dairy Federation*, 1997). Nitrate is still necessary, but the amount that needs to be added can be far smaller, e.g. 2.5 g rather than 15 g nitrate per 100 kg of milk (Walstra *et al*., 2006), with positive consequences for both the environment and consumer health.

In the 1990s bactofugation was applied to remove spores (mainly *Bacillus cereus* which is a major spoilage organism of pasteurized milk) from liquid drinking milk and was one of the first technologies used to produce extended-shelf-life milk. It is still a method in use, although newer methods have begun to replace it. The gain in shelf-life of fresh, pasteurized milk is about 3–5 days (still with the taste of normal pasteurized milk). For example, this is applied by Campina to guarantee extended shelf-life of various types of fresh drinking milk (te Giffel *et al.*, 2006). With hermetic bactofugation, the dairies are then able to add a few more days of life to their milk products. This satisfies both the consumers' demand for fresh product, which lasts longer once it has left the shop, and the dairy industry, since the consolidation of milk processing in a small number of very large dairies has led to a lengthening of the distribution chain.

Bactofugation is also beneficial in the production of UHT milk, concentrates and powders. Used in line, before the homogenization and UHT treatment, it allows a reduction in the high-heat temperature by about 15°C to obtain UHT products of at least equivalent bacteriological quality. Bactofugation can also be used to reduce the levels of spores in whey protein concentrates, infant formulae

and milk powder where application of sufficient heat to inhibit spores is not possible due to its effect on functionalities of milk components.

15.2.4   Configurations

There are several configurations of a bactofuge plant depending on its application, but the bactofugation is always combined with a thermal process and can be linked to existing pasteurization equipment.

Normally, the milk is first preheated in a heat exchanger (to 55–65ºC), giving a useful shelf-life increase of 5–15 days, depending on storage temperature. It is then separated and standardized to the desired fat content to achieve the fat-in-dry-matter content required. The standardized milk is then passed through a bactofuge (or a series of two bactofuges). In the case of two bactofuges, the bacteria-reduced milk is bactofuged again in a second bactofuge, and the bacterial concentrates of both bactofuges are mixed together. The concentrate can be discarded, or discharged separately for other suitable applications, but it is generally UHT-heated. The loss of casein micelles (perhaps as much as 6% of the total casein) will actually cause a decrease in cheese yield that may be avoided by supplementing the casein content (e.g. by adding ultrafiltration retentate) or by heat-sterilizing the bactofugate and returning it to the milk. Since the bactofugate is very concentrated, part of the bacteria-reduced milk is mixed with it before being pumped to a sterilizer in order to inactivate the spore-formers. Heat treatment is generally for a few seconds at 130ºC by steam infusion. During the manufacture of any cheese variety, the heated bactofugate is cooled by remixing with the bacteria-reduced milk before being pasteurized and cooled again before further processing.

The bactofugation is usually applied to remove bacteria, spores and, to a lesser extent, somatic cells from products that are pasteurized and, therefore, allows the heat treatment for decontaminating milk to be minimized. The final product, however, still contains heat-resistant bacteria and a significant number of spores, since the decimal reduction does not exceed 1.8. If complete removal of organisms is desired, this method becomes expensive.

15.3   Microfiltration (MF)

An alternative to bactofugation for the removal of microorganisms and somatic cells is the use of microfiltration (MF), which has met with some success. This method is particularly adapted to the removal of bacteria from skimmed milk, as the size of the microorganisms is in the same range as fat globules (see Fig. 15.1).

The first membrane developments for the separation of milk components occurred in the late 1960s with the advent of membrane separation, and has spawned a new industry for whey treatment as well as new avenues for cheese-making. Since then, membrane equipment has been adopted throughout the

Fig. 15.1   Approximate particle sizes for which separation by means of membrane filtration can be applied. Fundamentally, reverse osmosis, nanofiltration, and to a lesser extend ultrafiltration, do not separate on a particle-size basis. The size of some milk components is also indicated in comparison with membrane pore size range. The classical range of transmembrane pressures is mentioned for each membrane operation.

dairy processing chain, including milk reception, cheese-making, whey protein concentration, fractionation of protein and effluent treatment. Nowadays, 40% of the food applications of membrane processing are developed in the dairy industry, with applications and equipment serving as references for the industries treating other food liquids.

15.3.1   General aspects

Membrane processes are applied to separate a liquid into two fractions of different composition. The liquid is enclosed in a system confined by a semi-permeable membrane. The components passing the semi-permeable membrane constitute the *permeate*. The retained fraction is called the *retentate* (or concentrate).

   In the dairy industry, membrane filtration processes operate in crossflow mode (see Fig. 15.2). As opposed to dead-end filtration, in which the feed is pumped directly towards the filter and only the permeate leaves the membrane, the crossflow mode refers to the direction of the feed stream, which is applied tangentially over the surface of the membrane. This mode of operation makes it possible to sweep rejected solutes away from the membrane and influences back-transport of the accumulated solutes into the bulk of the feed (Zeman and Zydney, 1996; Cheryan, 1998). By comparison with the dead-end mode, the crossflow mode prevents sharp concentration build-up (also called concentration polarization) and membrane fouling. It thus improves filtration performance (permeation flux, J, and transmission of solute). Crossflow industrial plants are usually configured to operate continuously on a single or multistage recycle

Fig. 15.2   Crossflow and dead-end membrane filtration processes.
ΔP = transmembrane pressure.

basis. In this manner, the declining volume of retentate due to concentration proceeds in a separate stage where steps are taken to correct for the diminishing flow-rate and increasing viscosity.

In the case of membrane filtration, the driving force for the separation is a pressure difference over the membrane. This is called the transmembrane pressure, ΔP; it is generally realized by a pressure pump in the feed line and a valve in the retentate line. ΔP is classically defined and measured as the difference of pressure between the retentate and the permeate compartments according to:

$$\Delta P = \frac{P_{re} - P_{ro}}{P_p} \qquad\qquad 15.3$$

where P_{re} and P_{ro} are the pressure in the retentate (r) at the entrance (e) and at the outlet (o) of the membrane, respectively, and P_p is the pressure in the permeate.

Membranes and supporting structures (modules)
By definition, the membrane is a permeability-selective barrier that reduces the transfer of one solute compared to another one. In concentration mode, the membrane reduces the transfer of solutes by comparison with water. The membrane has a porous structure with pore size ranging from approximately 0.1 to 10 μm for microfiltration (MF), 1 nm to 0.1 μm for ultrafiltration (UF), a few nm for nanofiltration (NF), and a dense structure for reverse osmosis (RO) (see Fig. 15.1). It is notable that there are no precisely defined boundaries between the different operations, and that the higher the pore size, the lower the applied transmembrane pressure. Due to the wide range of membrane pore sizes, there are different ways of characterizing them. Microfiltration membranes are

usually characterized directly according to their mean pore diameter and pore size distribution. Due to the difficulty in measuring pore size of UF membranes, the concept of a nominal molar mass (molecular weight) cut-off (limit), MWCO, has been adopted. The MWCO of a membrane is defined as the molar mass of the solute that would be retained at 90% by the membrane. In RO and NF, membranes are usually characterized from their rejection values against mono- or divalent salt solutions.

Regardless of its material, the membrane is composed of two parts: a thick macroporous support layer ensuring the membrane mechanical resistance and a thin active layer attached to the support ensuring selectivity. Most membranes are made of polymers, mainly polysulfone and polyether-sulfone for UF and polyamide for NF and RO in the dairy industry. The currently used organic membranes can withstand high temperatures that are often limited to 50–60ºC due to the seals and adhesives of spiral-wound modules. They are also reasonably resistant to cleaning with acid and alkali, but altered by sodium hypochlorite solutions used as a disinfectant in most dairies (Bégoin *et al.*, 2006). Since the 1980s, there has been considerable interest in the use of ceramic membranes for dairy processing (mainly in MF applications) due to their very high thermal, chemical and disinfectant stability. These ceramic membranes, usually made from alumina, titanium oxide, zirconia or a mixture of both oxides, offer a narrow range of available pore sizes (mainly in the MF range) and tend to be considerably more expensive than their polymeric counterparts (typical costs for the ceramic membranes are in the order of $1500–3000/m^2 compared to the $70–300/m^2 for most polymeric membranes). These limitations have severely hindered the widespread acceptance of these ceramic membranes in most dairy applications. However, in the 1980s fouling problems limited the use of MF for bacteria removal in dairies (Holm *et al.*, 1986; Piot *et al.*, 1987) and the manufacture of new ceramic membranes comprising a multichannel geometry and a highly permeable support, associated with a new hydraulic concept (see below) allowed the development of industrial MF applications.

In practice, membranes are configured into modules, the design of which must satisfy a number of mechanical, hydrodynamic and economic requirements. A description of modules and their most important criteria are presented in Table 15.3. Currently, three main module types are applied in the dairy industry. Plates with flat membranes are still used but they are generally replaced now by spiral-wound membranes. Tubular systems are now exclusively used for ceramic membranes, and therefore mainly for microfiltration applications.

Efficiency of membrane separation processes
Several parameters characterize the efficiency of the process. The two most important parameters are the flow-rate of the permeate, or the permeation flux, J, and the membrane rejection, R.

The permeation flux, J, is usually presented in terms of volume per unit time per unit area ($L\,h^{-1}\,m^{-2}$) in order to allow a ready comparison of the performance of different membranes with different membrane areas:

Table 15.3   Comparison of different module configurations

Module configuration	Channel spacing (cm)	Packing density ($m^2 m^{-3}$)	Energy costs (pumping)	Particle plugging	Ease of cleaning
Tubular membrane (in hollow supporting tubes)	0.3–2.5	60	High	Low	Excellent
Flat membrane (on supporting plates)	0.03–0.25	300	Moderate	Moderate	Good
Spiral-wound membrane (membranes alternated with flexible supports wound around a tube)	0.03–0.1	600	Low	Very high	Poor to fair
Hollow fibre	0.02–0.25	1200	Low	High	Fair

Source: Zeman and Zydney (1996).

$$J = \frac{V_p}{At} = \frac{Q_p}{A} \qquad\qquad 15.4$$

where V_p is the permeate volume (m^3), Q_p the permeate flow-rate ($m^3 s^{-1}$), A the membrane area (m^2) and t the time necessary for the removal of V_p (s). In fact, J corresponds to the velocity of permeate passing the membrane and can be expressed in $m\,s^{-1}$.

Microfiltration is thought to behave like physical sieves, since the membranes are highly porous, and solvent and solute mass transfers are supposed to be mainly controlled by convective transport. The permeation flux, J, is then often modelled as a purely sieving process in terms of flow through a bundle of capillaries according to the Hagen–Poiseuille equation. In practice, this equation is complicated by properties of the membrane such as porosity or tortuosity effects and it is generally preferred to use Darcy's law:

$$J = \frac{\Delta P}{\eta_p R_m} = \frac{L_p \Delta P}{\eta_p} \qquad\qquad 15.5$$

where η_p is the viscosity of the permeate (Pa s), R_m (m^{-1}) is the membrane hydraulic resistance and L_p (m) is the permeability of the membrane. R_m and L_p depend in particular on membrane geometry (thickness, pore diameter, density of pores).

According to this equation, it is interesting to note that J depends on the operating parameter (ΔP), on the characteristics of the membrane (R_m or L_p) and on the characteristics of the fluid passing through the membrane (η_p).

With a solvent (such as water), J is proportional to the applied pressure, and the initial permeability of the membrane can be determined. This procedure is classically used in the industry for controlling the efficiency of cleaning procedures after production. However, during filtration of solutions containing macromolecules or dispersed components, the flux sharply decreases due to the

Fig. 15.3   Concentration gradient on a membrane along which a concentrating fluid flows: C_r, C_p, C_m = concentrations of a solute in the retentate, in the permeate and at the membrane surface, respectively; J = permeation flux ; ΔP = transmembrane pressure; δ = thickness of the limiting boundary layer, in which solutes are concentrated.

accumulation of retained solutes at the membrane surface (see Fig. 15.3). This phenomenon is known as polarization concentration. This strong concentration of the retained solutes can lead to several physico-chemical modifications at the membrane interface, mainly due to the surpassed solute solubility (gel formation, mineral precipitation, etc.) leading to membrane fouling. The concentration gradient and subsequent local modifications (fouling) affect both permeability and selectivity of the process. Generally, protein deposition and mineral (calcium phosphate) precipitation are major fouling agents during the membrane processing of dairy products. Fouling (R_f is defined as the hydraulic resistance due to fouling) adds another layer of resistance to that of the membrane, R_m (see equation 15.5), and diminishes filtration performance. A number of models are used to describe concentration polarization and fouling phenomena in both UF and MF processes. More details can be found in Zeman and Zydney (1996) and Cheryan (1998).

The rejection or retention of a solute by a membrane, R, is defined according to:

$$R = 1 - \frac{C_p}{C_r} \qquad\qquad 15.6$$

where C_p and C_r are the concentrations (in $kg\,m^{-3}$) of permeate and retentate, respectively. Based on this definition, if a component is completely rejected by the membrane, $C_p = 0$ and thus $R = 1$. On the other hand, for components which freely permeate the membrane, $C_p = C_r$ and $R = 0$.

This rejection, which varies during the course of the filtration time, is determined experimentally for a chosen solute in the permeate and retentate by sampling the fluids at the same time and analysing their composition. This is one of the particular ways of selecting the most appropriate membrane for a particular application.

Parameters affecting flux and rejection
In membrane filtration processes, the separation achieved depends on numerous parameters (Zeman and Zydney, 1996; Britz and Robinson, 2008). These include:

- Characteristics of the membrane and associated module (length, pore size distribution, materials, surface chemistry, etc.)
- Characteristics of the fluid to be treated (nature and physico-chemical characteristics of components, rheological behaviour, pH, ionic strength, etc.)
- Operating conditions (feed concentration, temperature, transmembrane pressure, crossflow velocity, v, or wall shear stress, τ_w, etc.), where τ_w represents the forces applied by the fluid flowing tangentially to the membrane on an element of the membrane area, and therefore characterizes the erosion at the membrane surface; it has been shown to be an effective parameter for characterizing the crossflow transport (Gésan-Guiziou *et al.*, 1999b).

Among operating parameters, ΔP and crossflow characteristics (either v or τ_w) are the main parameters influencing the performance. The dependence of J on ΔP is easy to understand since pressure is the driving force of the separation. J increases with increasing pressure, usually up to a limiting value (limiting flux) above which the permeate rate becomes pressure-independent, due to the accumulated layer or the gel formed at the membrane surface (see Fig. 15.4). Then, separation characteristics can be distinguished into two regions:

- The pressure-independent region, which should be avoided due to high fouling, difficulties of cleaning and high energy consumption. In that region, the increase in pressure, which is directly related to the pump consumption, does not lead to significant increase of flux.

Fig. 15.4 Influence of transmembrane pressure, ΔP, and wall shear stress at the membrane surface, τ_w on permeation flux, J; J_{lim} = limiting flux; P_{re} = pressure in the retentate at the membrane entrance; P_{ro} = pressure in the retentate at the outlet of the membrane.

- The pressure-dependent region, which should be favoured for optimal production.

Moreover, the limiting flux increases with increasing crossflow velocity or wall shear stress (see Fig. 15.4), due to increase of turbulence and erosion at the membrane surface and thinner deposit. Then, the balance between the convective force (ΔP) and erosion at the membrane surface (τ_w) makes it possible to design operations with reduced fouling, leading to longer production times (Gésan-Guiziou *et al.*, 1999a).

It is noticeable that in MF, crossflow velocity or wall shear stress is classically high in order to erode accumulated particles, and this leads to a high pressure drop ($P_{re} - P_{ro}$) in the retentate compartment. Then, in the case where the permeate pressure is constant, ΔP varies along the membrane length, from $\Delta P_e = P_{re} - P_p$ at the entrance to $\Delta P_o = P_{ro} - P_p$ at the outlet of the membrane. The difference between ΔP_e and ΔP_o, which corresponds to the retentate pressure drop, leads to fouling heterogeneity along the filtering path (see Fig. 15.4). Moreover, the low transmembrane pressure required for MF in order to avoid plugging of the membrane pores (see Fig. 15.1), can lead to negative transmembrane pressure at the membrane outlet (see Fig. 15.5).

To overcome fouling heterogeneity and make it possible to perform MF with simultaneous high crossflow velocity and low transmembrane pressure, most MF plants in dairies operate according to the hydraulic concept of the uniform transmembrane pressure (UTP) developed for bacterial removal of milk and patented by the Alfa-Laval Company under the trademark Bactocatch (Sandblom, 1974). The MF permeate is circulated in a co-current to the MF retentate in order to create a permeate pressure drop similar to the retentate pressure drop according to the following equation (see Fig. 15.5):

Fig. 15.5   Principle of the uniform transmembrane pressure system, numerical example: P = pressure; ΔP = transmembrane pressure; subscripts r = retentate; p = permeate; e = entrance; o = outlet.

$$\Delta P_e = \Delta P_o \Leftrightarrow P_{re} - P_{pe} = P_{ro} - P_{po}$$
$$\Leftrightarrow P_{re} - P_{ro} = P_{pe} - P_{po}$$
$$\Leftrightarrow \text{retentate pressure drop} = \text{permeate pressure drop}$$

In the method patented by Alfa-Laval, the permeate compartment is filled with plastic balls and is pressurized by pumping the permeate in a loop parallel to the direction of the retentate flow. In the module construction of Invensys APV to ensure UTP is different, the membranes are placed into small stainless steel tubes, without balls in order to reduce the external space between the housing and the membrane porous media.

The concentration of solutes is another key parameter that influences membrane separations. In industrial filtration, the concentration level is related to the volume reduction ratio, VRR, commonly called the volume concentration ratio (although the concentration of a volume remains unclear!). In discontinuous mode:

$$\text{VRR} = \frac{V_f}{V_r} \qquad\qquad 15.7a$$

where V_f and V_r are the volumes of feed and retentate, respectively. In continuous mode :

$$\text{VRR} = 1 + \frac{Q_p}{Q_r} \qquad\qquad 15.7b$$

where Q_p and Q_r are the flow-rates of permeate and retentate.

The permeation flux generally decreases when VRR increases due to accumulation of solutes at the membrane surface.

15.3.2   Application of microfiltration for the removal of bacteria, spores and somatic cells

In the 1990s, the total microfiltration area in the world dairy industry used for both the separation of casein micelles and the removal of microorganisms was less than $750\,m^2$ (van der Horst and Hanemaaijer, 1990). Today MF is an established technology for the removal of bacteria, spores and somatic cells in the dairy industry. The MF area can be estimated to have reached $15{,}000\,m^2$. This is far removed from ultrafiltration (membrane area around $350{,}000\,m^2$), which is the most widely used, mainly for milk and whey protein concentration, and nanofiltration, developed for whey demineralization.

Operating conditions
For bacterial removal by MF, the cream must be removed from milk before it can be microfiltered, because the distribution of the diameters of fat globules is similar to that of bacteria (see Fig. 15.1). The skimmed milk is then circulated continuously under pressure over a ceramic membrane with a pore size of $\sim\!1.4\,\mu m$ at a temperature between 35 and 55°C. The classical pore diameter of $1.4\,\mu m$ permits bacteria to be retained by the membrane to a large extent, while

performing almost complete permeation of all other milk components, mainly casein micelles. Membranes were originally configured in a monotube with an inner diameter ranging from 3 to 8 mm, but current designs use mostly the multichannel configuration with either classical cylindrical channels or various cross-sectional forms.

The homogeneous filtration conditions should be maintained throughout the filtering path, using either the uniform transmembrane pressure system (Bactocatch system, Fig. 15.5) or recent ceramic membranes with linear hydraulic resistance gradient. This new membrane concept makes it possible to obtain homogeneous filtration performance all along the membrane length without a permeate circulation loop and avoiding extra investment and running costs due to the permeate pump. The objective consisted in creating an inhomogeneous membrane, having a higher hydraulic resistance (R_m, equation 15.5) at the membrane entrance where the local transmembrane pressure is high, and a low resistance at the membrane outlet. Two types of membranes are commercially available. The first, known as Membralox GP (Garcera and Toujas, 1998) from Pall-Exekia, is based on a continuous variation of the porosity of the membrane support. The second, named Isoflux from Tami Industries, is based on continuous variation of the membrane layer thickness. Both membranes are obviously constructed for well-defined pressures and hydrodynamic conditions, and consequently must be used for well-defined applications.

Membranes that are most widely used for the removal of bacteria from milk are mainly Sterilox and GP (Pall-Exekia) and, to a lesser extent, Isoflux (Tami Industries). Tetra Pak and Invensys APV offer complete process lines in which MF is incorporated.

By combining high crossflow velocity (6–9 m s^{-1}) with a low transmembrane pressure ($\Delta P \approx 50$ kPa), the membranes lead to high fluxes ($J = 400$–650 L h^{-1} m^{-2}) for long operating periods, 10 h, and to low matter losses (5% of the skim milk stream at a volume reduction ratio VRR = 20, or 0.5% if a second MF process is incorporated with VRR = 200; see Fig. 15.6). Transmission rates of total solids, protein and fat of skimmed milk are 99.5, 99 and 63%, respectively (Maubois and Ollivier, 1997).

Skimmed milk MF makes it possible to decrease the microbial load of milk, while maintaining the organoleptic quality of milk due to low heat treatment (35–55ºC). MF was demonstrated to be more effective than bactofugation. With an MF using a 1.4 μm mean pore membrane, average decimal reduction of bacteria is good (see Table 15.4): the decimal reduction of bacteria ranged from 2–3 log with first ceramic membranes (see Table 15.4; Malmberg and Holm, 1988; Trouvé et al., 1991) and reaches 3–4 log with the currently used 1.4 μm Sterilox (announced to have a much narrower pore size distribution) or GP membranes, with no significant reduction in membrane performance efficiency. This means that microfiltered milk contains between 10 and 50 cfu mL^{-1}. The morphology of bacterial cells and cellular volume slightly influence the membrane retention properties. With 1.4 μm membranes, the observed decimal

Fig. 15.6   Schematic representation of process for microfiltration of whole milk. Dashed lines represent options for the treatment of retentate.

reduction in spore counts is high: retention from 99.1 to 99.99% (2–4 log) for both aerobic and anaerobic spores (te Giffel and van der Horst, 2004). Some authors have announced even higher spore decimal reductions, >3.5 log (99.98% of retention) or even >4.5 log (99.998% of retention), for spores such as *Bacillus cereus* (Olesen and Jensen, 1989) or *Clostridium tyrobutyricum* (Trouvé *et al.*, 1991). These high spore retention rates are probably due to binding of bacterial spores to part of the cell wall, resulting in larger apparent cell size (Maubois, 2002; Maubois and Schuck, 2005).

Decimal reduction of pathogenic bacteria (*Listeria monocytogenes*, *Brucella abortus*, *Salmonella* Typhimurium and *Mycobacterium tuberculosis*) is 3.5–4.0 (Saboya and Maubois, 2000). Such results ensure that microfiltered skimmed

Table 15.4   Approximate bacteria retention in skimmed milk microfiltration (1.4 μm MF membrane at a volume reduction ratio of 20)

Membrane	Membralox	Sterilox	Stérilox	GP Membrane
Membrane mean pore diameter (μm)	1.4	1.4	0.8	1.4
Crossflow velocity (m s^{-1})	7	7	na[a]	7
Permation flux (L h^{-1} m^{-2})	680	500	300-400	500
Decimal reduction of bacteria (–)	2–3 log	3–4 log	5–6 log	3–4 log

[a] na: not available.

milk contains less than $1\,cfu\,mL^{-1}$ of pathogenic bacteria, taking into account the usual contamination levels at farm level.

Somatic cells are evidently totally retained by the $1.4\,\mu m$ MF membrane (Saboya and Maubois, 2000; te Giffel and van der Horst, 2004) and, consequently, the microfiltered milk will not be degraded by their thermostable enzymes. The retention of somatic cells is also total with $5\,\mu m$ membrane and ranges from 93 to 99% with $12\,\mu m$ membrane (te Giffel and van der Horst, 2004). An average pore size of $5\,\mu m$ leads to milk without somatic cells but with fat content reduced by 17% (Maubois, 2002).

Stronger reductions can be obtained by using membranes with smaller pore size (0.5–$0.8\,\mu m$). Using a $0.5\,\mu m$ membrane, Lindquist (1998) showed an increase in bacteria removal up to 2 or even 3 logs compared to a $1.4\,\mu m$ membrane, with only a slight decrease of casein micelle permeation. Using a new membrane concept, Maubois (2008) found that decimal reduction of bacteria higher than 13 could be achieved.

Industrial applications

The process is schematically presented in Fig. 15.6, along with several options (indicated by dashed lines). The milk is skimmed before microfiltration. For production of a whole-milk product, the cream is separately heat-treated (heat treatments range from HTST pasteurization to UHT according to Maubois, 2002). The cream is classically treated at about 120ºC for 4 s to eliminate bacteria, including spore-formers, and added back to the microfiltered skimmed milk. The microfiltration retentate, which contains most of the bacteria and somatic cells and some large casein micelles, can be discharged separately for other suitable applications (such as animal feed after heat treatment). In order to reduce the volume of discharged retentate, a second MF stage can be added, to concentrate further the first retentate (Maubois and Schuck, 2005). In the Bactocatch process the retentate is blended continuously with cream and similarly heat-treated with the cream using a moderate UHT treatment (115–120ºC for 3 s). This procedure is, however, inadvisable, as thermostable enzymes still present in the retentate could actually lead to negative impacts on subsequent cheese quality (Maubois, 2002). In some other systems (the Invensys APV process), the retentate is fed back to the cream separator for repeated separation, where a significant number of bacteria, spores and somatic cells are removed with the separator sludge. The fraction of milk being heat-treated is then reduced (see Fig. 15.6).

The process has been commercialized for either drinking or cheese milks.

Several types of drinking milks are currently offered to consumers. In France, Marguerite® milk is considered to be raw milk because no pasteurization is applied. The microfiltered skimmed milk is mixed with the amount of heated cream requested for the fat standardization; the mixture is slightly homogenized and aseptically filled. The shelf-life of such milk is 15 days at 4–6ºC.

In most countries, to meet current regulatory requirements, whole milk produced using MF and intended for the drinking milk market undergoes a final high-temperature, short-time pasteurization step. Combined with a heat

treatment (72ºC, 15 s) and clean filling, the shelf-life of the product is greatly enhanced: microfiltered-pasteurized milk has a refrigerated shelf-life of 20–32 days, compared with 6–18 days for normal pasteurized milk. Compared with milk with a similar extended shelf-life obtained by a short-time heat treatment, it is considered to have a fresher flavour. However, part of the product, composed of fat globules, which generate the greater part of the sulfhydryl compounds on intense heat treatment (and retentate eventually), are sterilized.

Several tens of MF systems (10–20 $m^3 h^{-1}$) are currently running in Europe and in North America (in particular Canada) for the manufacture of drinking milk. In Europe, microfiltered-pasteurized milk is produced by several dairy companies (te Giffel *et al.*, 2006), e.g. by Parmalat and Granolaro in Italy. and by Arla as Cravendale PureFiltre in the UK. In all these countries, the microfiltered milks encountered great commercial success because of improvement in flavour and storage ability. In the UK, microfiltered milk with a shelf-life of 23 days has captured 11% of the market.

The high bacterial removal of the milk obtained with a 0.5 μm membrane makes possible the manufacture of milk with long shelf-life at room temperature using a moderate heat treatment (96ºC for 5 s) in order to inhibit endogenous enzymes. A commercial system, which then combines both microfiltration and moderate heat treatment, can produce sterile milk suitable for 12 months' ambient storage. Sensory tests show a flavour close to HTST classical milks and much better than the current UHT milks.

As well as being used for the drinking milk sector, MF pretreatment of skim milk can be expanded to all skim milk used for the production of milk derivatives such as low-heat milk powder, milk protein concentrates or micellar casein powder.

This process is also used for cheese milk to remove somatic cells and spores, particularly *Clostridium* species. By comparison with bactofugation, the high spore removal makes it possible to suppress the addition of nitrate, classically performed at 15 g per 100 kg to prevent the 'late blowing' of semi-hard and hard cheeses. Due to high retention of bacteria and spores, such a process is used by cheese-makers to produce safe raw milk cheeses. The MF pretreatment of milk can then be carried out either at 50ºC, as previously described, or at 35ºC with some specific adaptations of the running parameters in order to give the cheese-makers the possibility of avoiding all the detrimental effects of heat treatment on the non-fatty fraction of the used milk.

Due to the high bacteria and spore retention, the French regulatory authorities have permitted from 2002 the provisional use of MF milk for the making of Protected Designation of Origin (PDO) raw milk cheese (CNA, 2002). The permission stopped in 2007, but some cheeses are currently produced by MF, especially those using raw milk (*JO*, 2007): Camembert, for instance, can be produced from microfiltered milk without significant difference in flavour and texture compared to traditional cheese.

However, microfiltered milk has been described as 'too clean' by cheese-makers and prepared cheeses may lack flavour development as a result (Kelly *et*

al., 2008). The use of MF milk in cheese-making clearly raises the problem of how to make good quality cheese from ultra-clean cheese milk and what should be added in terms of the microbial ecosystem composition for obtaining typical ripening (and consequently the right organoleptic qualities) of the cheese variety produced. Further optimization is therefore required to achieve desirable characteristics of cheeses, and research must be conducted and adapted to each variety of cheese.

15.4   Conclusions

Like most foods, milk and its derivatives provide a favourable medium for spoilage microorganisms. Apart from heat treatments, bactofugation and microfiltration are the most widely used processes for lowering the bacteria, spore and somatic cell contents of milk and milk products. From an overview of published data it can be concluded that microfiltration is more efficient in removing bacteria and bacterial spores than bactofugation. Thus, membrane technology offers the dairy industry a powerful and flexible tool for a remarkable improvement of the hygienic status of milk and all dairy products.

The future of the use of this technology in the global dairy industry is very promising. The minimal heat treatment applied to the milk results in intact or at least little-damaged nutritional and bioactive properties of the milk components. This preservation method is therefore an appropriate tool for simultaneously increasing the safety of the product and preserving the nutritional and physico-chemical properties of the raw material. Due to the amount of research that has been focused on the characterization of milk components, on analytical methodologies and on separation processes, it is likely that the general quality and production efficiency of the various milk protein ingredients will increase in the near future. In that context, microfiltration could be a strategic step at the beginning of these future fractionation processes, limiting the cumulative effects of successive heat treatments.

In order to be totally accepted, some further developments should still be considered, in particular in terms of processing costs (high pumping costs, high thermal energy required, especially for membrane cleaning, etc.) and environmental burden (discharged water, single-use cleaning system, etc.). New microfiltration systems, to be integrated in cheese and milk processing systems, should also be more compact and space efficient. In this area some efforts could be focused on membranes/modules with reduced channel thickness, less fouling properties, and so on, in order to favour an increase in membrane compactness and a decrease in energy consumption. The wafer-stacked microsieves, having equal-sized pores and recently developed by a Dutch high-technology company named FluXXion, could offer new opportunities. Thanks to a specially designed crossflow system with continued back-pulsing, high permeabilities (10–100 times higher than those of classical ceramic membranes) could be reached.

In the future, and as it has been seen in the past with the difficulties encountered in making cheeses with satisfactory qualities through membrane separation processes, MF will give the opportunity to cheese scientists and technologists to acquire knowledge in numerous fields of dairy science (biochemistry, microbiology, etc.). For example, the removal of the entire contaminating flora by MF offers the possibility of studying how each type of starter bacteria added to the cheese milk acts on the ripening of cheeses. The possible removal of somatic cells also offers a means to study the consequences of somatic cells in many varieties of cheeses.

15.5   Sources of further information and advice

More detailed information about centrifugal separation can be found in:

- Mulder H and Walstra P (1974), *The Milk Fat Globule: Emulsion Science as Applied to Milk Products and Comparable Foods*, Wageningen, Pudoc, Chapter 8.

More detailed information about principles of cheese making can be found in:

- Walstra P, Wouters JTM and Geurts TJ (2006), *Dairy Science and Technology*, Boca Raton, FL, CRC Press, Taylor & Francis.

Two clear and comprehensive books, which emphasize basic aspects of all kinds of membrane processes but do not give much detail on dairy applications, are:

- Zeman LJ and Zydney AL (1996), *Microfiltration and Ultrafiltration: Principles and Applications*, New York, Marcel Dekker.
- Cheryan M (1998), *Ultrafiltration and Microfiltration Handbook*, Lancaster, PA, Technomic Publishing.

For enlightening studies of microfiltration applied to bacterial removal from dairy fluids:

- International Dairy Federation (1997), 'Implications of microfiltration on hygiene and identity of dairy products', *Bull Int Dairy Fed*, 320, 8–40.

More detailed information of membrane processes applied to dairy fluids in:

- Britz TJ and Robinson RK (2008), *Advanced Dairy Science and Technology*, Oxford, Blackwell Publishing.
- Mistry VV and Maubois JL (2004), 'Application of membrane separation technology to cheese production', in Fox PF, McSweeney PLH, Cogan TM and Guinee TP, *Cheese Chemistry, Physics and Microbiology. Vol 1 General Aspects*, London, Elsevier.

15.6   References

BÉGOIN L, RABILLER-BAUDRY M, CHAUFER B, FAILLE C, BLANPAIN-AVET P, BÉNÉZECH T and DONEVA T (2006), 'Methodology of analysis of a spiral-wound module. Application to PES membrane for ultrafiltration of skimmed milk', *Desalination*, 192(1–3), 40–53.

BRITZ TJ and ROBINSON RK (2008), *Advanced Dairy Science and Technology*, Oxford, Blackwell Publishing.

CNA, CONSEIL NATIONAL DE L'ALIMENTATION (2002), 'Avis du Conseil National de l'Alimentation sur l'emploi de lait microfiltré AOC', 25 June, http://www.agriculture.gouv.fr:alim/part/avis_39.pdf

CHERYAN M (1998), *Ultrafiltration and Microfiltration Handbook*, Lancaster, PA, Technomic Publishing.

DOYLE MP, GLASS KA, BEERY JT, GARCIA GA, POLLARD DJ and SCHULTZ RD (1987), 'Survival of *Listeria monocytogenes* in milk during high-temperature, short-time pasteurisation', *Applied Environ Microbiol*, 53, 1433–1438.

GARCERA D and TOUJAS E (1998), 'Macroporous support with permeability gradient and its manufacturing process', European Patent 0870534A1 FR 9704359 1–11.

GÉSAN-GUIZIOU G, BOYAVAL E and DAUFIN G (1999a), 'Critical stability conditions in crossflow microfiltration of skimmed milk: transition to irreversible deposition', *J Membrane Sci*, 158, 211–222.

GÉSAN-GUIZIOU G, DAUFIN G, BOYAVAL E and LE BERRE O (1999b), 'Wall shear stress: effective parameter for the characterization of the cross-flow transport in turbulent regime during skimmed milk microfiltration', *Lait*, 79, 347–354.

GRIFFITHS MW (1989), '*Listeria monocytogenes*: its importance in the dairy industry', *J Sci Food Agric*, 47, 133–158.

HOLM S, MALMBERG R and SVENSSON K (1986), 'Method and plant for producing milk with a low bacterial content', International Patent PCT No. 86/01687.

INTERNATIONAL DAIRY FEDERATION (1997), 'Implications of microfiltration on hygiene and identity of dairy products', *Bull Int Dairy Fed*, 320, 8–40.

JO, OFFICIAL JOURNAL OF THE FRENCH REPUBLIC (2007), Décret no. 2007-628 relatif aux fromages et spécialités fromagères, *JO*, 29 April 2007, Text no. 14.

KELLY AL, HUPPERTZ T and SHEEHAN JJ (2008), 'Pre-treatment of cheese milk: principles and developments', *Dairy Sci Technol*, 88, 549–572.

LINDQUIST A (1998), 'A method for the production of sterile skimmed milk', PCT Patent, WO No. 57549.

MALMBERG R and HOLM S (1988), 'Producing low-bacteria milk by microfiltration', *North Eur Dairy J*, 54, 30–32

MAUBOIS JL (2002), 'Membrane microfiltration: a tool for a new approach in dairy technology', *Aust J Dairy Technol*, 57, 92–96.

MAUBOIS JL (2008), 'Laits et produits laitiers en alimentation humaine. Apport des procédés technologiques', *Bull Acad Natle Méd*, 192(4), 703–711.

MAUBOIS JL and OLLIVIER G (1997), 'Extraction of milk proteins', in Damodaran S and Paraf A, *Foods Proteins and their Applications*, New York, Marcel Dekker, 579–595.

MAUBOIS JL and SCHUCK P (2005), 'Membrane technologies for the fractionation of dairy components', *Bull Int Dairy Fed*, 400, 2–7.

MISTRY VV and MAUBOIS JL (2004), 'Application of membrane separation technology to cheese production', in Fox PF, McSweeney PLH, Cogan TM and Guinee TP,

Cheese Chemistry, Physics and Microbiology. Vol 1 General Aspects, London, Elsevier.

MULDER H and WALSTRA P (1974), *The Milk Fat Globule: Emulsion Science as Applied to Milk Products and Comparable Foods*, Wageningen, Pudoc, Chapter 8.

OLESEN N and JENSEN F (1989), 'Microfiltration: the influence of operation parameters on the process', *Milchwissenschaft*, 44, 476–479.

PIOT M, VACHOT JC, VEAUX M, MAUBOIS JL and BRINKMAN GE (1987), 'Ecrémage et épuration bactérienne du lait entier cru par microfiltration sur membrane en flux tangentiel', *Tech Lait*, 1016, 42–46.

SABOYA LV and MAUBOIS JL (2000), 'Current developments of microfiltration technology in the dairy industry', *Lait*, 80, 541–553.

SANDBLOM RM (ALFA-LAVAL) (1974), 'Filtering process', Swedish Patent No. 7,416,257

TE GIFFEL MC and VAN DER HORST HC (2004), 'Comparison between bactofugation and microfiltration regarding efficiency of somatic cell and bacteria removal', *Bull Int Dairy Fed*, 389, 49–53.

TE GIFFEL MC, VAN ASSELT AJ and DE JONG P (2006), 'Shelf extension', *Dairy Ind Int*, 71(3), 44–49.

TROUVÉ E, MAUBOIS JL, PIOT M, MADEC MN, FAUQUANT J, ROUAULT A, TABARD J and BRINKMAN G (1991), 'Rétention de différentes espèces microbiennes lors de l'épuration du lait par microfiltration en flux tangentiel', *Lait*, 71, 1–13.

VAN DEN BERG G, DAAMEN CBG and STADHOUDERS J (1986), 'Bactofugation of cheese milk', *North Eur Food Dairy J*, 55, 63–68.

VAN DER HORST HC and HANEMAAIJER JH (1990), 'Crossflow microfiltration in the food industry: state of the art', *Desalination*, 77, 235–258.

WAES G and VAN HEDDEGHEM A (1990), 'Prevention of butyric acid fermentation by bacterial centrifugation of the chesse milk', *Bull Int Dairy Fed*, 251, 47–50.

WALSTRA P, WOUTERS JTM and GEURTS TJ (2006), *Dairy Science and Technology*, Boca Raton, FL, CRC Press, Taylor & Francis.

WIEKING W (2004), 'Removal of somatic cells from raw milk by centrifugal technology. State of the art', *Bull Int Dairy Fed*, 389, 45–47.

ZEMAN LJ and ZYDNEY AL (1996), *Microfiltration and Ultrafiltration: Principles and Applications*, New York, Marcel Dekker.

16

High pressure processing of milk

T. Huppertz, NIZO food research, The Netherlands

Abstract: High pressure (HP) processing is a non-thermal technology that involves treatment of a product at pressures ranging from 100 to 600 MPa. HP treatment of milk induces a myriad of changes, the most notable being the inactivation of micro-organisms and enzymes, the denaturation of whey proteins and the disruption of casein micelles. Although the process is comparatively expensive, it is of commercial interest because it achieves certain effects that cannot be achieved with current thermal processing technologies, e.g., the preservation of dairy products without denaturing bioactive proteins or desirable micro-organisms.

Key words: high pressure, milk, protein, micro-organism, enzyme, fat, minerals.

16.1   Introduction

The second half of the nineteenth century was an important period for the food industry, and the dairy industry in particular, as many processing technologies that are now commonplace in dairy factories worldwide were developed. In 1862, Louis Pasteur, together with Claude Bernard, completed their first test on the heat-induced inactivation of micro-organisms in food, a process soon afterwards referred to as pasteurization. Less than four decades later, in 1899, Auguste Gaulin was granted a patent for the design of the first prototype homogenizer, which was presented a year later at the 1900 Paris World Fair. Both technologies rapidly became indispensable tools in dairy processing. In the same time period, a publication arose from the West Virginia Experimental Agricultural Station (Hite, 1899) describing studies wherein a manually operated steel cylinder was used to pressurize milk to ~700 MPa and that such treatment

extended the shelf-life of raw milk by several days. Although this extension of shelf-life was a major achievement in those days, high pressure (HP) processing of milk and dairy products remained little more than an academic curiosity for almost another century, and has only recently proved to have commercially relevant applications for at least one of the largest dairy processors in the world. These applications are primarily aimed at niche and high-value markets, such as probiotic or immunoglobulin-rich dairy drinks and cheese spreads. Developments are unlikely to stop there, and more applications are expected in the future.

The aim of the material covered in this chapter is to provide the reader with an overview of HP processing of milk. For this purpose, the technology and principles of HP processing are first described in Section 16.2. Subsequently, the influence of HP processing on the constituents (Section 16.3) and microorganisms (Section 16.4) in milk will be evaluated. Finally, the implications of the aforementioned HP-induced changes in the constituents and microorganisms in milk will be discussed in relation to its shelf-life (Section 16.5) and processing characteristics (Section 16.6).

16.2   High pressure processing: principles and technologies

16.2.1   Principles of high pressure processing

HP processing of food products generally involves the treatment of products at a pressure in the range 100–1000 MPa (for conversions to other commonly used pressure units, see Table 16.1). In comparison, atmospheric pressure equals ~0.1 MPa, whereas the pressure exerted by the overlying water column, i.e., the hydrostatic pressure, at a depth of 10,000 metres in some of the deepest trenches of the ocean is approximately 100 MPa. It is this latter principle of pressure application, hydrostatic pressure, which is utilized in the pressure treatment of food products. Hydrostatic pressure here implies pressure exerted onto a product

Table 16.1   Commonly used pressure units

MPa	Bar	Atmospheres (atm)	Pounds per square inch (PSI)
100	1000	987	14,504
200	2000	1974	29,008
300	3000	2961	43,511
400	4000	3948	58,015
500	5000	4935	72,518
600	6000	5922	87,023
700	7000	6908	101,526
800	8000	7895	116,030
900	9000	8882	130,534
1000	10,000	9869	145,038

by the surrounding fluid, which in many cases is water. The main advantages of treating products with hydrostatic pressure are that the product reaches the pressure of the surrounding medium instantaneously and evenly and that pressure is distributed evenly throughout the product. As a result, the effectiveness of pressure treatment is independent of size or shape of the product, and uneven treatment throughout the product, which can occur on heat treatment, is avoided.

16.2.2   Technologies for high pressure processing

The basic technological requirements for HP processing of food products include a vessel of suitable strength, in which the treatment can be carried out, and a means of generating the pressure. The vessel is commonly produced from stainless steel and its wall thickness is determined primarily by the maximum pressure the system is required to maintain. The thickness of the vessel wall may be reduced by using a wire-wound or other prestressed design. Along with the vessel, a suitable closure is required to maintain pressure during processing. Hydrostatic pressure can be built up either by pumping additional fluid into the closed vessel or by compressing the fluid in the vessel using a piston. These principles are outlined in Fig. 16.1. In both cases, the mass of processing fluid per unit vessel volume is increased, until the desired pressure is reached.

The degree of compression required to reach the desired pressure depends on the compressibility of the processing fluid and product. Compressibility of water, the most commonly used processing fluid, is low; e.g., increasing pressure to 300 or 600 MPa reduces water volume by ~10% or ~15%, respectively. When pressure is built up rapidly and energy dissipation to the surroundings is (largely) prevented within the short time-frame, compressive, or adiabatic, heating is observed. For water, compressive heating is 2–3ºC per 100 MPa, but for fats and oils, compressive heating is considerably greater, i.e., 4–9ºC per 100 MPa (Rasanayagam *et al.*, 2003; Patazca *et al.*, 2007). Compressive heating of milk is marginally higher than that of water with only small differences observed between whole and skim milk (Patazca *et al.*, 2007; Buzrul *et al.*, 2008). A fat-rich dairy product like cream cheese, however, showed compressive heating of almost 5ºC per 100 MPa (Patazca *et al.*, 2007).

Equipment for HP processing can be divided into two categories, depending on its vessel volume. Research-scale HP units commonly have vessels ranging from several millilitres to several litres in volume. HP equipment for pilot- or production-scale processing is available with vessel volumes ranging from 35 to 600 L. Maximum pressure for production-scale HP processing equipment is currently limited to 600 MPa. Research units capable of reaching pressures >1000 MPa are available. Temperature control during HP treatment is also available, although for the larger HP units, i.e., >100 L vessel volume, temperature capabilities are often limited to temperatures in the range 5–30ºC. The two main manufacturers of production-scale HP equipment for processing of food products at present are US-based Avure Technologies (www.avure.com) and Spanish-based NC Hyperbaric (www.nchyperbaric.com).

Fig. 16.1   Schematic illustration of the principle of pressure build-up using a high pressure pump (a & b) or a piston (c & d).

In a typical HP processing cycle, the sample, which may be packaged, is placed in the vessel, which is already partially filled with processing fluid. The vessel is subsequently closed and the system is subsequently pressurized, as outlined above. When the desired pressure is reached, the sample is maintained at that pressure for the required holding time. This holding time may range from a few seconds up to several hours, although realistically, only holding times of under 10 minutes have commercial relevance. Following the holding time, pressure is released and the vessel can be unloaded. Overall, it is not uncommon for a pressure cycle with a 5 min holding time to require a total time of 15 min, as compression, decompression, loading and unloading of the vessel can each take several minutes. Several recent developments in equipment design have been aimed at increasing throughput, i.e., the vessel position has been changed from vertical to horizontal, which eliminates the need for a hoist or crane to load the product into the vessel and even offers the possibility of (semi-)automated loading and unloading of vessels. Furthermore, HP systems have been developed in which two or more vessels are placed in parallel, thereby increasing throughput because the process can be planned such that at least one vessel is at pressure at all times.

From the above it is apparent that HP processing is a process that, particularly for dairy operations, is carried out at a relatively small scale, with vessel

volumes of maximum 600 litres and cycle times taking up to 15 min in total. In addition, capital investment cost required for HP equipment still remains high, with processing-scale equipment costing several million euros. Taking into account operating and maintenance, cost estimates for HP treatment are 10–20 eurocents per kg of product, ~5–10-fold higher than for traditional thermal treatments (Van den Berg *et al.*, 2003). The result of this comparatively high cost, combined with the relatively low achievable throughput, leads to HP treatment being a potentially commercially viable option only for comparatively high-value products of low production volume. As outlined in the subsequent sections of this chapter, HP processing does indeed offer such opportunities even taking the aforementioned limitations into account.

16.3   Effect of high pressure on the constituents of milk

16.3.1   Basic physical and chemical considerations

In order to fully understand the effect of HP processing on milk constituents, it is first important to consider the effects of HP on basic physical and chemical equilibria which exist in milk and dairy products. When a product is treated with pressure, its equilibria are disturbed, and Le Chatelier's principle for such a situation states that: 'when a dynamic equilibrium is disturbed by changing the conditions, the position of equilibrium moves to counteract the change' (Atkins, 1995). Under pressure, where the volume of the system is reduced, the system will re-equilibrate by suppressing reactions that involve an increase in volume, while promoting those that involve a decrease in reaction. For liquid milk systems, the volume changes are primarily related to changes in the organization of water molecules around its constituents, rather than changes in the volume of the constituents themselves. The configuration of water molecules around the charged ions is considerably more compact than around the uncharged salts, as a result of which ionization reactions are characterized by a negative reaction volume and favoured under HP. This has, as outlined in Section 16.3.4, an impact on solubility of milk salts, as well as electrostatic interactions, either attractive or repulsive, between or within protein molecules. The volume changes associated with interactions of proteins are primarily due to changes in the compactness of arrangement of water around the proteins, rather than properties of the protein itself (Hvidt, 1975). This is exemplified by the association of casein, which is strongly affected by pressure; association of β-casein is at a minimum at ~150 MPa, above which it increases again (Payens and Heremans, 1969).

16.3.2   Effects of high pressure on lipids in milk

Compared to the research effort on HP-induced changes in milk salts and proteins, HP-induced changes in milk fat have received far less attention. Milk fat globules appear to remain intact under pressure, with no substantial HP-

induced changes in globule size being observed (Dumay *et al.*, 1996; Kanno *et al.*, 1998; Gervilla *et al.*, 2001; Huppertz *et al.*, 2003). HP-induced interaction of caseins and, in particular, whey proteins with the milk fat globule membrane are observed (Ye *et al.*, 2004). The physical properties of the milk lipids themselves are also affected by HP treatment. Lipid crystallization in HP-treated cream occurs at a higher temperature than in cream kept at atmospheric pressure (Buchheim and Abou El-Nour, 1992). Such effects have been related to a HP-induced shift in the solid/liquid transition temperature of milk fat to a higher value (Frede and Buchheim, 2000).

16.3.3   Effect of high pressure on carbohydrates in milk

Knowledge of the effect of high pressure on the predominant carbohydrate in milk is limited to date. Studies by Huppertz *et al.* (2004a) showed that degradation or hydrolysis of lactose did not occur under pressure. Effects of HP on other technologically relevant aspects of lactose in milk, e.g., its participation in the Maillard reaction, have not been studied to date.

16.3.4   Effect of high pressure on salts in milk

As outlined in more detail by Walstra *et al.* (2006), milk contains a wide variety of salts, the major ones being the phosphate, citrate, chloride and carbonate salts of sodium, potassium, calcium and magnesium. When milk is pressurized, mineral solubility, as outlined in Section 16.3.1, increases due to favoured solvation of ions. For the sodium and potassium salts, this is of limited importance as they are already fully soluble under physiological conditions. However, milk contains far more calcium and magnesium phosphate than is soluble under physiological conditions. The insoluble fraction of these salts is found in the casein micelles, and referred to as colloidal calcium phosphate (CCP) or micellar calcium phosphate (MCP). MCP contains ~70% of all calcium, 30% of all magnesium and 50% of inorganic phosphate in milk (Walstra *et al.*, 2006).

   In milk, and other liquid dairy products, increased solubility of salts under pressure is highlighted particularly by the increased solubility of MCP under high pressure (Hubbard *et al.*, 2002; Huppertz and De Kruif, 2007a). In unconcentrated milk at neutral pH and ambient temperature, solubilization of MCP increases with increasing pressure up to ~400 MPa, where all MCP is solubilized (Huppertz and De Kruif, 2007a). The HP-induced increase in calcium phosphate solubility is rapidly reversible on release of pressure, leading to the reversal of most of the calcium phosphate to the micellar state (Hubbard *et al.*, 2002). HP-treated milk has been reported to have higher levels of non-sedimentable calcium and phosphorus than untreated milk (Schrader and Buchheim, 1998; Lopez-Fandino *et al.*, 1998), but these can by no means be taken as a measure of HP-induced solubilization of MCP. Regnault *et al.* (2006) recently showed that levels of non-sedimentable calcium and inorganic phosphate were indeed higher in HP-treated milk than in untreated milk, but that levels of ultrafiltrable calcium

and inorganic phosphate are comparable in the same samples. Hence, it appears that the HP-induced increase in non-sedimentable calcium and inorganic phosphate in milk is caused by an increased level of protein-bound calcium and inorganic phosphate which is not sedimented. This increase is related to HP-induced changes in the casein micelles, which are covered in Section 16.3.5.

In addition to the aforementioned effect of HP on soluble calcium and inorganic phosphate under pressure and in HP-treated milk, small increases in the concentration of ionic calcium in milk have been observed immediately post HP-treatment (Lopez-Fandino *et al.*, 1998; Zobrist *et al.*, 2005). However, these increases have been observed to revert readily on subsequent storage post-HP treatment, even in a matter of minutes at above-ambient temperature (Zobrist *et al.*, 2005). Although thus far not examined, it is by no means unreasonable to assume that the considerable adiabatic cooling that occurs on decompression is largely responsible for HP-induced increases in calcium ion activity and that re-equilibration of temperature subsequently results in rapid re-equilibration of calcium ion equilibria.

16.3.5   Effect of high pressure on proteins in milk

Of all the constituents of milk, the milk proteins have achieved most attention in terms of investigations of HP-induced changes therein. Since caseins and whey proteins are distinctly different protein classes, they will be treated separately in this section. These areas have been reviewed in detail recently by Huppertz *et al.* (2002, 2006a, b), Lopez-Fandino (2006a, b) and Considine *et al.* (2007).

Effect of high pressure on caseins

The caseins are a class of four gene-products, denoted α_{s1}-, α_{s2}-, β- and κ-caseins, which represent ~80% of all protein in bovine milk (Swaisgood, 2003). Caseins are characterized by little secondary and tertiary structure, which is at least partially related to the high level of proline residues (De Kruif and Holt, 2003). As a result of their 'natively unfolded' or 'rheomorphic' structure, caseins are not significantly affected by HP treatment. However, the caseins in milk exist predominantly in the form of association colloids, called casein micelles. The structure and stability of casein micelles are maintained by strong interactions of phosphorylated serine residues of the caseins with nanoclusters of amorphous calcium phosphate, and interactions between the caseins are a result of cooperative weak interactions, which include, but are not limited to, hydrogen bonding, hydrophobic interactions and electrostatic interactions (De Kruif and Holt, 2003). Studies have shown that micellar integrity is reduced if the MCP is solubilized or cooperative attraction between proteins is suppressed (De Kruif and Holt, 2003; Smiddy *et al.*, 2006).

Under pressure, casein micelles are disrupted, as is visualized by decreases in turbidity and increases in light transmission (Kromkamp *et al.*, 1996; Huppertz and De Kruif, 2006, 2007b; Huppertz *et al.*, 2006c; Orlien *et al.*, 2006) with *in-situ* measurements. This micellar disruption process is time-dependent, its extent

increasing with holding time, although at higher pressures, e.g. >300 MPa, maximum disruption is often already reached during the pressurization phase. The extent of micellar disruption increases with pressure up to ~400 MPa (Huppertz *et al.*, 2006c; Orlien *et al.*, 2006) and is probably a result of solubilization of MCP (Huppertz and De Kruif, 2006), which also reaches a maximum at ~400 MPa for unconcentrated milk. Micellar disruption under HP is greater at a lower temperature (Gebhart *et al.*, 2005; Orlien *et al.*, 2006), lower pH (Huppertz and De Kruif, 2006) or lower concentration of milk solids (Huppertz and De Kruif, 2006), all of which again largely relate back to the influence of temperature, pH and milk solids concentration on the solubility of calcium phosphate. The presence of whey proteins does not affect the disruption of casein micelles under pressure (Huppertz and De Kruif, 2007b). A summary of the factors affecting HP-induced disruption of casein micelles under pressure is given in Table 16.2.

Table 16.2 Factors affecting the disruption of casein micelles under high pressure

Factor	Observed effect	Reference
Treatment conditions		
Pressure	Disruption increases with increasing pressure	Kromkamp *et al.* (1996); Huppertz *et al.* (2006c); Orlien *et al.* (2006); Gebhart *et al.* (2005)
Time	Disruption increases with increasing time	Kromkamp *et al.* (1996); Huppertz *et al.* (2006c); Orlien *et al.* (2006)
Temperature	Disruption decreases with increasing temperature	Orlien *et al.* (2006); Gebhart *et al.* (2005)
Milk properties		
pH	Disruption decreases with increasing pH	Huppertz and De Kruif (2006)
Milk solids	Disruption decreases with increasing milk solids content	Huppertz and De Kruif (2006)
Additives		
Whey protein	No effect	Huppertz and De Kruif (2007b)
Calcium chloride	Disruption decreases on addition of calcium chloride	Huppertz and De Kruif (2006)
Sodium phosphate	Disruption increases on addition of sodium phosphate	Huppertz and De Kruif (2006)
Sodium chloride	Disruption increases on addition of sodium chloride	Huppertz and De Kruif (2006)
Pretreatment of milk		
Enzymatic crosslinking	Disruption decreases with increasing extent of crosslinking	Huppertz and Smiddy (2008)

Table 16.3 Factors affecting the reformation of casein particles during prolonged high pressure treatment

Factor	Observed effects	References
Treatment conditions		
Pressure	Reformation occurs only at 250 and 300 MPa	Huppertz *et al.* (2006c); Orlien *et al.* (2006)
Time	Reformation increases with increasing treatment time	Huppertz *et al.* (2006c); Huppertz and De Kruif (2006); Orlien *et al.* (2006)
Temperature	Reformation is more extensive at a higher temperature	Orlien *et al.* (2006)
Milk properties		
pH	Reformation is less extensive at a higher pH	Huppertz and De Kruif (2006)
Additives		
Whey protein	No effect	Huppertz and De Kruif (2007b)
Calcium chloride	Rate of reformation decreases on addition of calcium chloride	Huppertz and De Kruif (2006)
Sodium phosphate	Little effect on reformation	Huppertz and De Kruif (2006)
Sodium chloride	Rate of reformation increases on addition of sodium chloride	Huppertz and De Kruif (2006)
Pretreatment of milk		
Enzymatic crosslinking	Crosslinking reduces the rate of micellar aggregation	Huppertz and Smiddy (2008)

If micellar disruption is not complete, some reversal of HP-induced decreases in turbidity and increases in light transmission is observed during prolonged treatment. Such reversal is indicative of aggregation of micellar fragments and occurs on treatment at 200–300 MPa (Huppertz *et al.*, 2006c; Huppertz and De Kruif, 2006, 2007b; Orlien *et al.*, 2006) and is more extensive at higher temperature (Orlien *et al.*, 2006). Other factors influencing the reassociation process are outlined in Table 16.3. Following release of pressure, which is accompanied by a reduction in the solubility of calcium phosphate (Hubbard *et al.*, 2002), HP-induced increases in turbidity largely reverse, indicating reformation of casein particles; following treatment at >300 MPa, however, reversibility is not complete and part of the increase in light transmission, or decrease in turbidity, remains (Kromkamp *et al.*, 1996; Huppertz *et al.*, 2006c).

The combined effects of compression, holding under pressure and decompression result in casein micelles in HP-treated milk often having physicochemical properties that differ considerably from those of untreated milk; a review thereof is provided by Huppertz *et al.* (2002, 2006a, b) and Lopez-Fandino *et al.* (2006a). Both HP-induced increases and decreases in micelle size have been observed, the former being generally observed after treatment at intermediate pressures, e.g. 200–300 MPa, whereas the latter occurs predominantly at pressures

Table 16.4   Factors affecting casein micelle size in HP-treated milk

Factor	Influence	References
Treatment conditions		
Pressure	Little effect up to 200 MPa; treatment at 250 MPa increases micelle size; treatment at $\geq$300 MPa decreases micelle size	Needs *et al.* (2000a); Huppertz *et al.* (2004b, c); Regnault *et al.* (2004); Anema *et al.* (2005)
Time	Increases in micelle size at longer treatment time at 250 MPa	Huppertz *et al.* (2004b)
Temperature	Increasing temperature enhances increases in micelle size at 250 MPa; little effect on disruption at higher pressures	Gaucheron *et al.* (1997); Huppertz *et al.* (2004b); Anema *et al.* (2005)
Milk properties		
pH	Greater increases in micelle size of milk with higher pH	Huppertz *et al.* (2004b)
Milk concentration	Greater increase in micelle size at 300 MPa with milk concentration; smaller decrease in micelle size at 400–600 MPa	Anema (2008)
Additives		
Whey protein	Little or no influence on increases in casein micelle size	Regnault *et al.* (2004); Gaucheron *et al.* (1997); Anema *et al.* (2005); Huppertz *et al.* (2004b)
Pretreatment of milk		
Enzymatic crosslinking	Prevents HP-induced micellar disruption	Smiddy *et al.* (2006)
Preheating milk	No effect on HP-induced changes in micelle size	Huppertz *et al.* (2004c)

>300 MPa (Gaucheron *et al.*, 1997; Huppertz *et al.*, 2004b, c; Regnault *et al.*, 2004; Anema *et al.*, 2005). Both increases and decreases in micelle size are strongly influenced by factors such as treatment time, temperature, milk pH and various additives, the influence of which are summarized in Table 16.4. As a result of the HP-induced changes in the casein micelles, some processing characteristics of milk are altered significantly, as outlined in Section 16.6.

Effect of high pressure on whey proteins
The whey proteins, which represent ~20% of total milk protein in bovine milk, are globular proteins and the major proteins in this class are α-lactalbumin (α-la), β-lactoglobulin (β-lg), blood serum albumin (BSA) and the immuno-globulins A (IgA), G (IgG) and M (IgM). Their globular structure makes the

whey proteins, unlike the caseins, susceptible to heat- and pressure-induced denaturation. Of the whey proteins, β-lg is the least stable to pressure, and tends to unfold at pressures >100 MPa; α-la is considerably more stable to pressure, with denaturation generally observed only at pressures >400 MPa (for reviews see Huppertz *et al.*, 2006a; Lopez-Fandino, 2006b; Considine *et al.*, 2007). As a result of the unfolding, the reactive free sulphydryl-group of β-lg becomes exposed and can subsequently undergo irreversible sulphydryl–disulphide interchange reactions, with (1) other β-lg molecules; (2) whey proteins other than β-lg; (3) cysteine-containing caseins; (4) proteins of the milk fat globule membrane; or (5) other cysteine-containing milk proteins, e.g. plasmin. The degrees to which these interactions occur depend on the accessibility of the disulphide groups in the proteins. HP-induced denaturation of whey proteins depends on a wide variety of factors, as outlined in Table 16.5. In skim milk, most HP-denatured whey protein is associated with the casein micelles (Huppertz *et al.*, 2004b), but in whole milk, a significant proportion of denatured whey protein also associates with the milk fat globule membrane (Ye *et al.*, 2004). HP-induced denaturation and association of whey proteins affect cheese- and yoghurt-making properties of milk, as outlined in Section 16.6.

One class of whey proteins of particular interest with respect to HP processing is the immunoglobulins. Immunoglobulins have been attributed nutritional and therapeutic potential, but are rapidly denatured during conventional thermal processing required to inactivate bacteria. The immunoglobulins are, however, considerably more stable to HP processing, with ~90% of colostral IgG surviving treatment at 500 MPa for 5 minutes (Indyk *et al.*, 2008). Such

Table 16.5   Factors affecting HP-induced denaturation of whey proteins in milk

Factor	Influence	References
Pressure	Increasing pressure >100 MPa increases denaturation of β-lg Increasing pressure >400 MPa increases denaturation of α-la	Lopez-Fandino *et al.* (1996); Huppertz *et al.* (2004b, d)
Temperature	HP-induced denaturation of β-lg and α-la increases with increasing temperature	Lopez-Fandino & Olano (1998); Huppertz *et al.* (2004b)
Time	HP-induced denaturation of β-lg and α-la increases with increasing time	Lopez-Fandino *et al.* (1996); Huppertz *et al.* (2004b, d)
pH	HP-induced denaturation of β-lg and α-la is more extensive at a higher pH	Arias *et al.* (2000); Huppertz *et al.* (2004b)
Micellar calcium phosphate (MCP)	HP-induced denaturation of β-lg and α-la is less extensive in the absence of micellar calcium phosphate	Huppertz *et al.* (2004d)

effects allow opportunities for the preservation of immunoglobulin-rich products by HP, with minimal loss of activity.

HP treatment can also modify whey protein structure in a manner which provides access for proteases to cleavage of sites which are inaccessible in the native structure of the protein. Subsequent proteolysis, which may occur either during or after HP treatment, can be beneficial in terms of reducing protein allergenicity or yielding potential bioactive peptides. In its native form bovine β-lactoglobulin is extremely stable to digestion by pepsin and chymotrypsin (Reddy *et al.*, 1988; Breiteneder and Mills, 2005) and contributes significantly to the overall allergenicity of bovine milk (Besler *et al.*, 2002). As high pressure treatment ≥ 100 MPa unfolds β-lg, exposing the normally buried proteolytic cleavage sites, improved digestibility of this protein during or after high pressure treatment has been investigated as a means of reducing or eliminating the allergenicity (Olsen *et al.*, 2003; Chicon *et al.*, 2006; Zeece *et al.*, 2008). However, it is important to note that improved digestibility of β-lg does not necessarily imply reduced allergenicity, since hydrolysis products may still be allergenic (Wroblewska *et al.*, 2004; Zeece *et al.*, 2008); specific studies are necessary to investigate this further. The utilization of high pressure-induced changes in protein structure to expose cleavage sites for proteolytic enzymes with the aim of obtaining bioactive peptides from milk proteins has received little or no attention thus far, but may be of interest for future studies.

16.3.6   Effect of high pressure on enzymes in milk

Most indigenous milk enzymes are quite baroresistant, with resistance to treatment at pressure up to 400 MPa being observed for plasmin (Garcia-Risco *et al.*, 2000, 2003; Scollard *et al.*, 2000a, b; Huppertz *et al.*, 2004e), alkaline phosphatase (Lopez-Fandino *et al.*, 1996; Rademacher *et al.*, 1998; Ludikhuyze *et al.*, 2000), lactoperoxidase (Lopez-Fandino *et al.*, 1996; Ludikhuyze *et al.*, 2001), xanthine oxidase (Olsen *et al.*, 2004), phosphohexoseisomerase (Rademacher *et al.*, 1998), γ-glutamyltransferase (Rademacher *et al.*, 1998; Pandey and Ramaswamy, 2004) and lipase (Pandey and Ramaswamy, 2004). In contrast, acid phosphatase activity in milk was reduced considerably on treatment at a pressure ≥ 200 MPa (Balci *et al.*, 2002).

The high stabilities of plasmin and lipase to HP processing in milk can be of some concern in terms of utilization of HP-treated milk in various applications. The lipid fraction in homogenized milk, for instance, is extremely susceptible to lipolysis and most products prepared from homogenized milk, i.e., milk for consumption, yoghurt or ice cream, require complete inactivation of lipase, which is commonly achieved by heat treatment (Walstra *et al.*, 2006). If such heat treatment is replaced by HP treatment to achieve particular benefits, additional measures need to be in place to ensure adequate lipase inactivation. Likewise, plasmin can play an important role in age-gelation of UHT-sterilized milk products (Kelly and McSweeney, 2003), and achieving high degrees of inactivation of plasmin is required to provide adequate stability.

16.4   Effects of high pressure on micro-organisms in milk

As outlined in Section 16.1, the first reported study on HP treatment of milk (Hite, 1899) was performed with the aim of improving the microbial shelf-life through bacterial inactivation. Since then, numerous studies have investigated the HP-induced inactivation of the microflora naturally present in milk, as well as of micro-organisms purposely inoculated into milk. HP inactivates micro-organisms via several mechanisms, e.g., damaging bacterial cell walls and membranes, inhibiting bacterial protein synthesis (Landau, 1967), destroying ribosomes (Gross et al., 1993), inactivating intracellular enzymes (Balci and Wilbey, 1999) or altering yeast morphology (Osumi et al., 1992; Kobori et al., 1995) and genetic mechanisms (Hoover et al., 1989). Sub-lethal damage of cells, resulting in a loss of viability, has been reported following HP treatment (McClements et al., 2001; Ritz et al., 2002). This may lead to an overestimation of numbers inactivated by the treatment and, during storage of treated foods, sublethally damaged cells may be repaired and multiply, causing spoilage or the presence of pathogenic bacteria in the food product.

   HP-induced inactivation of micro-organisms in milk is, as is the case for other products and other processing technologies, influenced by a myriad of factors related to the micro-organisms themselves and the medium in which they are suspended. First of all, the type of micro-organism has a large effect on the degree of inactivation achieved by HP treatment. In general, yeasts and moulds are most susceptible to HP treatment, with most being inactivated by treatment at 200–400 MPa. Vegetative bacteria require more severe treatment, i.e. 300–600 MPa, to reach a desirable degree of inactivation, whereas bacterial spores require even higher pressure, e.g. 600–1000 MPa, often in combination with temperatures >80ºC for inactivation. The low barostability of yeasts and moulds is exploited in a patented application to extend the shelf-life of probiotic yoghurt (Carroll et al., 2004). By using probiotic strains with a high resistance to pressure, HP can be used to inactivate all yeasts and moulds in yoghurt, as a result of which a product with a shelf-life up to 3 months can be achieved.

   Within each class of micro-organisms, large species-dependent differences in barostability are observed; furthermore, even for a particular species, large differences between subspecies may be observed. Barostability of bacteria in milk appears higher when they are in the stationary, rather than the exponential growth phase (Isaacs et al., 1995; McClements et al., 2001; Caseidi et al., 2002; Hayman et al., 2007). Furthermore, bacterial growth at above-ambient temperature (35ºC) or in the cold (10ºC) yields more-barostable bacteria than growth at near-ambient conditions (10–30ºC) (Caseidi et al., 2002; Hayman et al., 2007). The influence of growth temperature on bacterial barostability may be related to the growth-temperature-induced changes in the composition of the bacterial membrane and the uptake of protective osmolytes (Hayman et al., 2007).

   The medium in which micro-organisms are HP-treated also affects their barostability. Much fundamental work on HP-induced inactivation of micro-organisms has been performed on buffered model systems, but when the

outcomes therefrom are compared to actual inactivation in food systems, micro-organisms are often more stable in food products than in buffer systems. Factors which affect baroprotection include water activity and pH (Jordan *et al.*, 2001; Wouters *et al.*, 1998), the presence of antimicrobial compounds (Black *et al.*, 2005; Masschalck *et al.*, 2000), as well as individual food constituents, such as fat, proteins or solutes (Molina-Hoppner *et al.*, 2004) or minerals (Cheftel, 1995; Hauben *et al.*, 1998; Van Opstal *et al.*, 2003). Milk also exerts a strong baroprotective effect over buffered systems (Styles *et al.*, 1991; Patterson *et al.*, 1995; Simpson and Gilmour, 1997; Black *et al.*, 2007). This baroprotective effect of milk is, at least in the case of *Listeria monocytogenes*, strongly related to the mineral fraction in milk, more particularly the MCP, which, when solubilized under HP, can provide additional buffering and whose solubilized divalent cations may stabilize the cell membrane against HP-induced damage (Black *et al.*, 2007). Due to the aforementioned poor translation of results obtained in model systems to those actually observed in milk, only the latter will be discussed in this section.

Table 16.6 shows an overview of the extent of HP-induced inactivation that has been observed for the different classes of the natural microflora in milk. Treatment at a pressure <400 MPa does not appear to result in excessive reductions in the microflora of milk (Table 16.6) and certainly does not appear to offer any major advantages over conventional pasteurization techniques. To obtain a significant reduction (>4 log units) in the level of naturally present microbes in raw milk, treatment at 600 MPa is required. One further striking aspect of the data presented in Table 16.6 is that in most studies outlined herein, rather long treatment times are used. As achievable throughput is likely to be a major limitation for commercialization of HP processing for milk, further study on whether significant inactivation can also be achieved with shorter treatment times, e.g. <10 min, is required. A further aspect that should be considered here is that sublethal damage of cells, resulting in a loss of viability, has been reported following HP treatment of milk (McClements *et al.*, 2001; Hayman *et al.*, 2007; Bull *et al.*, 2005). This may lead to an overestimation of numbers inactivated by the treatment and, during storage of treated foods, sublethally damaged cells may be repaired and multiply, causing spoilage or the presence of pathogenic bacteria in the food product. Koseki *et al.* (2008) reported that the application of a mild heat treatment, e.g. 5 min at 50ºC, could prevent any regrowth of HP-inactivated *Listeria monocytogenes* in milk. Further studies on this phenomenon for the complete microflora of milk appear warranted.

Table 16.7 provides an extensive overview of the level of HP-induced inactivation that can be achieved for selected micro-organisms inoculated into milk. Although it is obvious that there is a high variation in baroresistance among species and strains, direct comparison is difficult due to the differences between treatment conditions, milk types, levels of inoculation, and strains used. However, it is apparent that Gram-positive species such *Listeria monocytogenes* and *Staphylococcus aureus* and Gram-negative *Escherichia coli* are the most baroresistant of the species investigated in milk.

Table 16.6 High pressure-induced inactivation of indigenous bacteria in raw bovine milk. Inactivation data given correspond to the minimum conditions required to obtain the highest level of inactivation reported in the respective publications

Bacterial class	Treatment conditions			Initial level (log cfu mL^{-1})	Inactivation (log cfu mL^{-1})	References
	Pressure (MPa)	Time (min)	Temp. (ºC)			
Total microflora	350	20	?	6.0	<3.0	Mussa and Ramaswamy (1997)
Total microflora	300	60	?	6.0	3.0	Mussa *et al.* (1999)
Total microflora	700	30	13	6.0	3.5	Timson and Short (1965)
Aerobes	400	30	25	3.5	1.0	Garcia-Risco *et al.* (1998)
Aerobes	600	25	25	~7.0	~7.0	Dogan and Erkmen (2004)
Aerobes	400	30	25	4.5	1.0	Lopez-Fandino *et al.* (1996)
Aerobic mesophiles	600	5	55	?	4.5	Nabhan (2004)
Psychrotrophs	200	30	25	3.2	1.0	Lopez-Fandino *et al.* (1996)
Psychrotrophs	400	30	25	2.5	2.5	Garcia-Risco *et al.* (1998)
Enterobacteriaceae	400	30	25	3.0	3.0	Garcia-Risco *et al.* (1998)

Table 16.7 High pressure-induced inactivation of exogenous bacteria in milk. Inactivation data given correspond to the minimum conditions required to obtain the highest level of inactivation reported in the respective publications

Micro-organism	Treatment conditions			Inactivation (log cfu mL^{-1})	References
	Pressure (MPa)	Time (min)	Temp. (ºC)		
Escherichia coli	400	15		6.0	Isaacs *et al.* (1995)
Escherichia coli O157:H7 NCTC 12079	600	30	20	2.0	Patterson *et al.* (1995)
Escherichia coli O157:H7 NCTC 12079	200	15	60	8.0	Patterson and Kilpatrick (1998)
Escherichia coli O157:H7 NCTC 12079	700	15	40	8.0	Patterson and Kilpatrick (1998)
Escherichia coli MG1655	600	15	20	3.0	Garcia-Graells *et al.* (1999)
Escherichia coli NCTC 11601	600	30	20	7.0	Linton *et al.* (2001)
Escherichia coli NCTC 9706	600	30	20	7.0	Linton *et al.* (2001)
Escherichia coli 19-2017	600	10	20	6.7	Linton *et al.* (2001)
Escherichia coli MC1061	500	5	20	6.5	Black *et al.* (2005)
Listeria monocytogenes Scott A	344	60	23	6.0	Styles *et al.* (1991)
Listeria monocytogenes Scott A	300	60	20	2.0	Mussa *et al.* (1999)
Listeria monocytogenes Scott A	600	10	20	7.5	Chen and Hoover (2004)
Listeria monocytogenes NCTC 11994	375	30	20	1.0	Patterson *et al.* (1995)
Listeria monocytogenes NCTC 11994	375	30	20	1.5	Simpson and Gilmour (1997)
Listeria monocytogenes 11994	400	24	8	6.0	McClements *et al.* (2001)

Listeria monocytogenes Scott A	400	24	8	6.0	McClements *et al.* (2001)
Listeria monocytogenes	375	30	20	5.0	Simpson and Gilmour (1997)
Listeria monocytogenes 4a KUEN 136	600	16	25	7.0	Dogan and Erkmen (2004)
Listeria monocytogenes	550	25	5	7.0	Koseki *et al.* (2008)
Listeria innocua 4202	500	5	20	3.8	Black *et al.* (2005)
Listeria innocua 4202	500	5	20	3.0	Black *et al.* (2007)
Staphylococcus aureus NCTC 10652	600	30	20	5.0	Patterson *et al.* (1995)
Staphylococcus aureus ATCC 27690	350	6	20	8.6	Erkmen and Karatas (1997)
Staphylococcus aureus As 1.2465	330	15	34	6.0	Gao *et al.* (2005)
Bacillus cereus NCFB 578	400	15	8	6.0	McClements *et al.* (2001)
Bacillus cereus NCFB 1031	400	18	8	5.5	McClements *et al.* (2001)
Pseudomonas fluorescens ANA11	250	18	8	5.5	McClements *et al.* (2001)
Pseudomonas fluorescens NCDO 1524	250	18	8	6.0	McClements *et al.* (2001)
Pseudomonas fluorescens M114	300	5	20	8.2	Black *et al.* (2005)
Yersinia enterocolitica	400	10	20	7.5	De Lamo-Castellvi *et al.* (2005)
Mycobacterium avium ssp. *paratuberculosis*	500	10	20	4.0	Lopez-Pedemonte *et al.* (2006)
Mycobacterium avium ssp. *paratuberculosis*	600	5	20	6.5	Donaghy *et al.* (2007)
Salmonella typhimurium	600	20	21	6.0	Guan *et al.* (2005)

16.5   Shelf-life of high pressure-treated milk

Despite the great amount of studies carried out on the effect of HP on micro-organisms which are either naturally present or purposely inoculated into milk, only few have looked at the shelf-life of HP-treated milk. When HP is carried out at $\geq$500 MPa for 5 min at <4°C or >55°C, the refrigerated shelf-life of raw milk is prolonged to 21 days (Nabhan, 2004). Raw milk pressurized at 400 MPa for 30 min at 25°C contained <7 log psychrotrophs ml^{-1} after storage for 45 days at 7°C, whereas unpressurized milk contained >7 log of these bacteria after only 15 days, indicating the significant extension of shelf-life achievable by high pressure treatment (Garcia-Risco *et al.*, 1998). HP treatment has also been shown to increase the shelf-life of pasteurized milk (Adams *et al.*, 2006). However, as pasteurization is so effective for milk preservation and micro-organisms have such varying sensitivities to pressure treatment, and may be more resistant when treated in milk, a very clear benefit needs to be demonstrated before pressure treatment can realistically compete with pasteurization for safe, large-scale, milk production.

Except for the microbial shelf-life, the physicochemical shelf-life of HP-treated milk is also crucial for potentially marketing HP-treated dairy products. Key aspects in this respect are the undesirable creaming and gelation of milk. Gelation of milk, which may for instance occur in UHT-treated milk products or sterilized concentrated milk products, e.g. evaporated sugared condensed milk (Walstra *et al.*, 2006), has not been studied in HP-treated milk to date. Plasmin-induced proteolysis has been attributed a key role in the age-gelation of UHT treated milk products (Kelly and McSweeney, 2003). Proteolysis of caseins by plasmin is promoted following treatment at 200–400 MPa, which is probably a combined result of the enzyme withstanding treatment at such pressure and the disruption of casein micelles facilitating access of the proteinase to its substrate (Scollard *et al.*, 2000a; Huppertz *et al.*, 2004e). Hence, there may be some cause for concern when using HP treatment for long shelf-life milk products and additional measures for plasmin inactivation may need to be implemented. Creaming, however, is affected by HP treatment: treatment at 100–300 MPa promotes the creaming of unhomogenized milk, whereas treatment at 400–800 MPa suppresses it (Huppertz *et al.*, 2003). Unfortunately, the HP-induced suppression of creaming is by no means sufficient to eliminate the requirement for homogenization.

16.6   Processing characteristics of high pressure-treated milk

The aforementioned effects of HP treatment on the constituents and properties of milk have considerable implications for the characteristics of dairy products derived therefrom. Of particular interest therefore are the HP-induced changes in the milk proteins, as these, to a large extent, determine the physicochemical properties and stability of popular dairy products, such as cheese and yoghurt. Such effects are briefly outlined below.

HP-induced changes in milk proteins and other milk constituents have considerable effects on cheese-making properties of milk. These effects were reviewed by Lopez-Fandino (2006a) and Stewart *et al.* (2006), and only a brief summary is provided here. The time required for rennet-induced coagulation of milk to commence, i.e. the rennet coagulation time (RCT), is reduced by treatment at 100–400 MPa, with a minimum at 250–300 MPa, but steadily increases at pressures >400 MPa (Lopez-Fandino *et al.*, 1996; Needs *et al.*, 2000a; Zobrist *et al.*, 2005). Such effects appear to be related to two counteracting mechanisms, the predominance of which differs with pressure. HP-induced reductions in RCT are caused by micellar disruption, whereas HP-induced association of whey proteins with the casein micelles increases RCT. At relatively low pressures, the influence of micellar disruption on RCT predominates, whereas whey protein denaturation is the predominant factor at higher pressures (Zobrist *et al.*, 2005). HP-induced decreases in RCT are generally accompanied by HP-induced increases in the rate of firming and final firmness of the rennet-induced coagulum, which also reach maxima for milk treated at 250–300 MPa (Lopez-Fandino *et al.*, 1996; Needs *et al.*, 2000a; Zobrist *et al.*, 2005). The yield of curd increases with increasing treatment pressure, which can be attributed to incorporation of whey proteins in the curd as well as increased moisture retention in the curd (Lopez-Fandino *et al.*, 1996; Huppertz *et al.*, 2004f). A further interesting application in this area is the use of a short HP treatment to restore the rennet coagulation characteristics of heated milk (Huppertz *et al.*, 2005). Translation of the aforementioned data derived on model systems to actual cheese is currently lacking, as is detailed information on the ripening, flavour and texture of cheese produced from HP-treated milk.

HP treatment of milk also affects the yoghurt-making properties of milk. The rate of acidification during the manufacture of yoghurt is higher in HP-treated milk than in milk treated at high temperatures (85°C for 20 min: Needs *et al.*, 2000b). Moreover, acid-induced gelation of HP-treated milk occurs at higher pH (Desobry-Banon *et al.*, 1994; Gervilla *et al.*, 2001) and yoghurt manufactured from HP-treated milk exhibits less syneresis (Harte *et al.*, 2003), increased incorporation of whey proteins (Needs *et al.*, 2000b), and increased gel strength (Desobry-Banon *et al.*, 1994; Needs *et al.*, 2000b; Gervilla *et al.*, 2001; Harte *et al.*, 2002) compared to that made from unpressurized milk. Needs *et al.* (2000b), using transmission electron microscopy, observed that micelles in yoghurt manufactured from HP-treated milk were smooth-surfaced particles that formed densely packed strands, while in yoghurt manufactured from heat-treated milk, micelles were separated by dense filamentous projections at their surfaces.

16.7   Future trends

The extensive research effort summarized in the previous sections highlights that HP treatment has considerable, and in some cases unique effects on milk. However, it is also important to keep in mind that, compared to other techno-

logies, particularly thermal processing, HP processing has some considerable disadvantages, i.e., it is an expensive processing technique for the dairy industry, and has a comparatively limited scale. As such, only applications whereby the use of HP allows the manufacturer to reach desirable product properties or functionalities that cannot be achieved by traditional means offer commercial potential. Several recent applications have been identified and patented and are close to being commercialized. New Zealand's Fonterra has been a front runner in this respect, having patented applications for the use of HP in producing lactoferrin-enriched jellies, and preservation of colostrum and probiotic drinks and yoghurts. It is conceivable that further applications in the field of bioactives and probiotics are likely to be discovered soon. Furthermore, the aforementioned use of HP to produce hypoallergenic, bioactive milk protein hydrolysates is clearly an area which deserves further attention.

16.8   References

ADAMS E G, DELWICHE J F and HARPER W J (2006), 'Effects of high pressure processing on the microbiological and physical properties of pasteurized fluid milk products', *Milchwissenschaft*, 61, 277–280.

ANEMA S G (2008), 'Effect of milk solids concentration on whey protein denaturation, particle size changes and solubilization of casein in high-pressure-treated skim milk', *Int Dairy J*, 18, 228–235.

ANEMA S G, LOWE E K and STOCKMAN G (2005), 'Particle size changes and casein solubilisation in high-pressure-treated skim milk', *Food Hydrocolloid*, 19, 257–267.

ARIAS M, LOPEZ-FANDINO R and OLANO A (2000), 'Influence of pH on the effects of high pressure on milk proteins', *Milchwissenschaft*, 55, 191–194.

ATKINS P W (1995), *Concepts in Physical Chemistry*, Oxford, Oxford University Press.

BALCI A T and WILBEY R A (1999), 'High-pressure processing of milk – the first 100 years in the development of a new technology', *Int J Dairy Technol*, 52, 149–155.

BALCI A T, LEDWARD D A and WILBEY R A (2002), 'Effect of high pressure on acid phosphatase in milk', *High Pressure Res*, 22, 639–642.

BESLER M, EIGENMANN P and SCHWARTZ R H (2002), 'Cow's milk (*Bos domesticus*)', *Internet Symp Food Allergens*, 4, 19–106.

BLACK E P, KELLY A L and FITZGERALD G F (2005), 'The combined effect of high pressure and nisin on inactivation of microorganisms in milk', *Innov Food Sci Emerg Technol*, 6, 286–292.

BLACK E P, HUPPERTZ T, FITZGERALD G F and KELLY A L (2007), 'Baroprotection of vegetative bacteria by milk constituents: a study of *Listeria innocua*', *Int Dairy J*, 17, 104–110.

BREITENEDER H and MILLS E N C (2005), 'Molecular properties of food allergens', *J Allergy Clin Immun*, 115, 14–23.

BUCHHEIM W and ABOU EL-NOUR A M (1992), 'Induction of milkfat crystallization in the emulsified state by high hydrostatic pressure', *Fat Sci Technol*, 94, 369–373.

BULL M K, HAYMAN M M, STEWART C M, SZABO E A and KNABEL S J (2005), 'Effect of prior growth temperature, type of enrichment medium, and temperature and time of

storage on recovery of *Listeria monocytogenes* following high pressure processing of milk', *Int J Food Microbiol*, 101, 53–61.

BUZRUL S, ALPAS H, LARGETEAU A, BOZOGLU F and DEMAZEAU G (2008), 'Compression heating of selected pressure transmitting fluids and liquid foods during high hydrostatic pressure treatment', *J Food Eng*, 85, 466–472.

CARROLL T, CHEN P, HARNETT M and HARNETT J (2004), 'Pressure treating food to reduce spoilage', International Patent, publication number WO 2004/032655 A1.

CASEIDI, M A, MANAS P, NIVEN G, NEEDS E and MACKEY B M (2002), 'Role of membrane fluidity in pressure resistance of *Escherichia coli* NCTC 8164', *Appl Environ Microbiol*, 68, 5965–5972.

CHEFTEL J C (1995), 'Review: High pressure, microbial inactivation and food preservation', *Food Sci Technol Int*, 1, 75–90.

CHEN H and HOOVER D G (2004), 'Use of Weibull model to describe and predict pressure inactivation of *Listeria monocytogenes* Scott A in whole milk', *Innov Food Sci Emerg Technol*, 5, 269–276.

CHICON R, LOPEZ-FANDINO R, QUIROS A and BELLOQUE J (2006), 'Changes in chymotrypsin hydrolysis of *β*-lactoglobulin A induced by high hydrostatic pressure', *J Agric Food Chem*, 54, 2333–2341.

CONSIDINE T, PATEL H A, ANEMA S G, SINGH H and CREAMER L K (2007), 'Interactions of milk proteins during heat and high hydrostatic pressure treatments: a review', *Innov Food Sci Emerg Technol*, 8, 1–23.

DE KRUIF C G and HOLT C (2003), 'Casein micelle structure, functions and interactions', in Fox P F and McSweeney P L H, *Advanced Dairy Chemistry, Volume 1: Proteins*, New York, Kluwer Academic/Plenum Publishers, 233–276.

DE LAMO-CASTELLVI S, ROIG-SAUGES A X, CAPELLAS M, HERNANDEZ-HERRERO M and GUAMIS, B (2005), 'Survival and growth of *Yersinia enterocolitica* strains inoculated in skimmed milk treated with high hydrostatic pressure', *Int J Food Microbiol*, 102, 337–342.

DESOBRY-BANON S, RICHARD F and HARDY J (1994), 'Study of acid and rennet coagulation of high pressurized milk', *J Dairy Sci*, 77, 3267–3274.

DOGAN C and ERKMEN O (2004), 'High pressure inactivation kinetics of *Listeria monocytogenes* inactivation in broth, milk, peach and orange juices', *J Food Eng*, 62, 47–52.

DONAGHY J A, LINTON M, PATTERSON M F and ROWE M T (2007), 'Effects of high pressure and pasteurization on *Mycobacterium avium* ssp. *paratuberculosis* in milk', *Lett Appl Microbiol*, 45, 154–159.

DUMAY E, LAMBERT C, FUNTENBERGER S and CHEFTEL J C (1996), 'Effects of high pressure on the physico-chemical characteristics of dairy creams and model oil/water emulsions', *Lebensm Wissenschaft Technol*, 29, 606–625.

ERKMEN O and KARATAS S (1997), 'Effect of high hydrostatic pressure on *Staphylococcus aureus* in milk', *J Food Eng*, 33, 257–262.

FREDE E and BUCHHEIM W (2000), 'The influence of high pressure upon the phase transition behaviour of milk-fat and milk-fat fractions', *Milchwissenschaft*, 55, 683–686.

GAO Y L, WANG Y X and JIANG H H (2005), 'Effect of high pressure and mild heat on *Staphylococcus aureus* in milk using response surface methodology', *Process Biochem*, 40, 1849–1854.

GARCIA-GRAELLS C, MASSCHALCK B and MICHIELS C W (1999), 'Inactivation of *Escherichia coli* in milk by high-hydrostatic-pressure treatment in combination with

antimicrobial peptides', *J Food Protect*, 62, 1248–1254.

GARCIA-RISCO M R, CORTES E, CARRASCOSA A V and LOPEZ-FANDINO R (1998), 'Micro-biological and chemical changes in high pressure treated milk during refrigerated storage', *J Food Protect*, 61, 735–737.

GARCIA-RISCO M R, OLANO A, RAMOS M and LOPEZ-FANDINO R (2000), 'Micellar changes induced by high pressure. Influence in the proteolytic activity and organoleptic properties of milk', *J Dairy Sci*, 83, 2184–2189.

GARCIA-RISCO M R, RECIO I, MOLINA E and LOPEZ-FANDINO R (2003), 'Plasmin activity in pressurized milk', *J Dairy Sci*, 86, 728–734.

GAUCHERON F, FAMELART M H, MARIETTE F, RAULOT K, MICHEL F and LE GRAET Y (1997), 'Combined effects of temperature and high-pressure treatments on physicochemical characteristics of skim milk', *Food Chem*, 59, 439–447.

GEBHART R, DOSTER W and KULOZIK U (2005), 'Pressure-induced dissociation of casein micelles: size distribution and effects of temperature', *Braz J Med Biol Res*, 38, 1209–1214.

GERVILLA R, FERRAGUT V and GUAMIS B (2001), 'High hydrostatic pressure effects on color and milk fat globule size in ewe's milk', *J Food Sci*, 66, 880–885.

GROSS M, LEHLE K, JAENICKE R and NIERHAUS K H (1993), 'Pressure induced dissociation of ribosomes and elongation cycle intermediates: Stabilising conditions and identification of the most sensitive functional state', *Eur J Biochem*, 218, 463–468.

GUAN D, CHEN H and HOOVER D G (2005), 'Inactivation of *Salmonella typhimurium* DT104 in UHT whole milk by high hydrostatic pressure', *Int J Food Microbiol*, 104, 145–153.

HARTE F, AMONTE M, LUEDECKE L, SWANSON B and BARBOSA-CÁNOVAS G V (2002), 'Yield stress and microstructure of set yogurt made from high hydrostatic pressure-treated full fat milk', *J Food Sci*, 67, 2245–2250.

HARTE F, LUEDECKE L, SWANSON B AND BARBOSA-CÁNOVAS G V (2003), 'Low-fat set yogurt made from milk subjected to combinations of high hydrostatic pressure and thermal processing', *J Dairy Sci*, 86, 1074–1082.

HAUBEN K J A, BERNAERTS K and MICHIELS C W (1998), 'Protective effect of calcium on inactivation of *Escherichia coli* by hydrostatic pressure', *J Appl Microbiol*, 85, 678–684.

HAYMAN M M, ANANTHESWARAN R S and KNABEL S J (2007), 'The effects of growth temperature and growth phase on the inactivation of *Listeria monocytogenes* in whole milk during high pressure processing', *Int J Food Microbiol*, 115, 220–226.

HITE B H (1899) 'The effect of high pressure in the preservation of milk', *W VA Agr Exp St Bull*, 58, 15–35.

HOOVER D G, METRICK C, PAPINEAU A M, FARKAS D F and KNORR D (1989), 'Biological effects of high hydrostatic pressure on food microorganisms', *Food Technol*, 43, 99–107.

HUBBARD C D, CASWELL D, LÜDEMANN H D and ARNOLD M (2002), 'Characterisation of pressure-treated skimmed milk powder dispersions: application of NMR spectroscopy', *J Sci Food Agr*, 82, 1107–1114.

HUPPERTZ T and DE KRUIF C G (2006), 'Disruption and reassociation of casein micelles under high pressure: influence of milk serum composition and casein micelle concentration', *J Agric Food Chem*, 54, 5903–5909.

HUPPERTZ T and DE KRUIF C G (2007a), 'High pressure-induced solubilisation of micellar calcium phosphate from cross-linked casein micelles', *Colloid Surface A*, 295, 1–3, 264–268.

HUPPERTZ T and DE KRUIF C G (2007b), 'Disruption and reassociation of casein micelles during high pressure treatment: influence of whey proteins', *J Dairy Res*, 74, 194–197.

HUPPERTZ T and SMIDDY M A (2008), 'Behaviour of partially cross-linked casein micelles under high pressure', *Int J Dairy Technol*, 61, 51–55.

HUPPERTZ T, FOX P F and KELLY A L (2002), 'Effects of high pressure on constituents and properties of milk', *Int Dairy J*, 12, 561–572.

HUPPERTZ T, FOX P F and KELLY A L (2003), 'High pressure-induced changes in creaming properties of bovine milk', *Innov Food Sci Emerg Technol*, 4, 349–359.

HUPPERTZ T, FOX P F and KELLY A L (2004a), 'Influence of high pressure treatment on acidification of bovine milk by lactic acid bacteria', *Milchwissenschaft*, 59, 249–252.

HUPPERTZ T, FOX P F and KELLY A L (2004b), 'High pressure treatment of bovine milk: effects on casein micelles and whey proteins', *J Dairy Res*, 71, 97–106.

HUPPERTZ T, FOX P F and KELLY A L (2004c), 'Properties of casein micelles in high pressure-treated bovine milk', *Food Chem*, 87, 103–110.

HUPPERTZ T, FOX P F and KELLY A L (2004d), 'High pressure-induced denaturation of α-lactalbumin and β-lactoglobulin in bovine milk and whey: a possible mechanism', *J Dairy Res*, 71, 489–495.

HUPPERTZ T, FOX P F and KELLY A L (2004e), 'Plasmin activity and proteolysis in high pressure-treated bovine milk', *Lait*, 84, 297–304.

HUPPERTZ T, FOX P F and KELLY A L (2004f), 'Effect of high pressure on the yield of cheese-curd from bovine milk', *Innov Food Sci Emerg Technol*, 5, 1–8.

HUPPERTZ T, HINZ K, ZOBRIST M R, UNIACKE T, KELLY A L and FOX P F (2005), 'Effects of high pressure treatment on the rennet coagulation and cheese-making properties of heated milk', *Innov Food Sci Emerg Technol*, 6, 279–285.

HUPPERTZ T, FOX P F, DE KRUIF C G and KELLY A L (2006a), 'High pressure-induced changes in bovine milk proteins: a review', *Biochim Biophys Acta*, 1764, 593–598.

HUPPERTZ T, SMIDDY M A, UPADHYAY V K and KELLY A L (2006b), 'Effects on high pressure on bovine milk: a review', *Int J Dairy Technol*, 59, 58–66.

HUPPERTZ T, KELLY A L and DE KRUIF C G (2006c), 'Disruption and reassociation of casein micelles under high pressure', *J Dairy Res*, 73, 294–298.

HVIDT A A (1975), 'Discussion of pressure–volume effects in aqueous protein solutions', *J Theor Biol*, 50, 245–252.

INDYK H E, WILLIAMS J W and PATEL H A (2008), 'Analysis of denaturation of bovine IgG by heat and high pressure using an optical biosensor', *Int Dairy J*, 18, 359–366.

ISAACS N S, CHILTON P and MACKEY B (1995), 'Studies on the inactivation by high pressure of micro-organisms', in Ledward D A, Johnston D E, Earnshaw, R G and Hasting A P M, *High Pressure Processing of Foods,* Nottingham University Press, Nottingham, UK, 65–79.

JORDAN S L, CASCUAL C, BRACEY E and MACKEY B M (2001), 'Inactivation and injury of pressure-resistant strains of *Escherichia coli* O157 and *Listeria monocytogenes* in fruit juices', *J Appl Microbiol*, 91, 463–469.

KANNO C, UCHIMURA T, HAGIWARA T, AMETANI M and AZUMA N (1998), 'Effect of hydrostatic pressure on the physicochemical properties of bovine milk fat globules and the milk fat globule membrane', in Isaacs N S, *High Pressure Food Science, Bioscience and Chemistry*, Cambridge, The Royal Society of Chemistry, 182–192.

KELLY A L and MCSWEENEY P L H (2003), 'Indigenous proteinases in milk', in Fox P F and McSweeney P L H, *Advanced Dairy Chemistry, Volume 1: Proteins*, New York,

Kluwer Academic/Plenum Publishers, 495–521.

KOBORI H, SATO M, TAMEIKE A, HAMADA K, SHIMADA S and OSUMI M (1995), 'Ultrastructural effects of pressure stress to the nucleus in *Saccharomyces cerevisiae*: a study by immunoelectron microscopy using frozen thin sections', *FEMS Microbiol Lett*, 132, 253–258.

KOSEKI S, MINUZO Y and YAMAMOTO K (2008), 'Use of milk-heat treatment following high pressure processing to prevent recovery of pressure-injured *Listeria monocytogenes* in milk', *Food Microbiol*, 25, 288–293.

KROMKAMP J, MOREIRA R M, LANGEVELD L P M and VAN MIL P J J M (1996), 'Microorganisms in milk and yoghurt: selective inactivation by high hydrostatic pressure', in *Heat Treatments and Alternative Methods*, Brussels, International Dairy Federation, 266–271.

LANDAU J (1967), 'Induction, transcription and translation in *Escherichia coli*: A hydrostatic pressure study', *Biochim Biophys Acta*, 149, 506–512.

LINTON M, MCCLEMENTS J M J and PATTERSON M F (2001), 'Inactivation of pathogenic *Escherichia coli* in skimmed milk using high hydrostatic pressure', *Innov Food Sci Emerg Technol*, 2, 99–104.

LOPEZ-FANDINO R (2006a), 'High pressure-induced changes in milk proteins and possible applications in dairy technology', *Int Dairy J*, 16, 1119–1131.

LOPEZ-FANDINO R (2006b), 'Functional improvement of milk whey proteins by high pressure treatment', *Crit Rev Food Sci Nutr*, 46, 351–363.

LOPEZ-FANDINO R and OLANO A (1998), 'Effects of high pressures combined with moderate temperatures on the rennet coagulation properties of milk', *Int Dairy J*, 8, 623–627.

LOPEZ-FANDINO R, CARRASCOSA A V and OLANO A (1996), 'The effects of high pressure on whey protein denaturation and cheese-making properties of raw milk', *J Dairy Sci*, 79, 929–936.

LOPEZ-FANDINO R, DE LA FUENTE M A, RAMOS M and OLANO A (1998), 'Distribution of minerals and proteins between the soluble and colloidal phases of pressurized milks from different species', *J Dairy Res*, 65, 69–78.

LOPEZ-PEDEMONTE T, SEVILLA I, GARRIDO J M, ADURIZ G, GUAMIS B, JUSTE R A and ROIG-SAGUES A X (2006), 'Inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in cow's milk by means of high hydrostatic pressure at mild temperatures', *Appl Environ Microbiol*, 72, 4446–4449.

LUDIKHUYZE L, CLAEYS W and HENDRICKX M (2000), 'Combined pressure–temperature inactivation of alkaline phosphatase in bovine milk: a kinetic study', *J Food Sci*, 65, 155–160.

LUDIKHUYZE L R, CLAEYS W L and HENDRICKX M E (2001), 'Effect of temperature and/or pressure on lactoperoxidase activity in bovine milk and acid whey', *J Dairy Res*, 68, 625–637.

MASSCHALCK B, GARCIA-GRAELLS C, VAN HAVER E and MICHIELS C W (2000), 'Inactivation of high pressure resistant *Escherichia coli* by lysozyme and nisin under high pressure', *Innov Food Sci Emerg Technol*, 1, 39–47.

MCCLEMENTS J M J, PATTERSON M F and LINTON M (2001), 'The effect of growth stage and growth temperature in high hydrostatic pressure inactivation of some psychrotrophic bacteria in milk', *J Food Protect*, 64, 514–522.

MOLINA-HOPPNER A, DOSTER W, VOGEL R F and GANZLE M (2004), 'Protective effect of sucrose and sodium chloride for *Lactococcus lactis* during sublethal and lethal high-pressure treatments', *Appl Environ Microbiol*, 70, 2013–2020.

MUSSA D M and RAMASWAMY H S (1997), 'Ultra high pressure processing of milk. Kinetics of microbial destruction and changes in physicochemical characteristics', *Lebensm Wissenschaft Technol*, 30, 551–557.

MUSSA D M, RAMASWAMY H S and SMITH J P (1999), 'High pressure (HP) destruction kinetics of *Listeria monocytogenes* Scott A in raw milk', *Food Res Int*, 31, 343–350.

NABHAN M A (2004), 'Proteolyse et redistribution des proteins entre différentes fractions azotes après traitment du lait par hautes pressions', Thesis, Institut National Polytechnique de Lorraine, France.

NEEDS E C, STENNING R A, GILL A L, FERRAGUT V and RICH G T (2000a), 'High-pressure treatment of milk: effects on casein micelle structure and on enzymic coagulation', *J Dairy Res*, 67, 31–42.

NEEDS E C, CAPELLAS M, BLAND A P, MANOJ P, MACDOUGAL D and PAUL G (2000b), 'Comparison of heat and pressure treatments of skim milk, fortified with whey protein concentrate, for set yoghurt preparation: effects on milk proteins and gel structure', *J Dairy Res*, 67, 329–348.

OLSEN K, KRISTIANSEN K R and SKIBSTED L H (2003), 'Effect of high hydrostatic pressure on the steady-state kinetics of tryptic hydrolysis of beta-lactoglobulin', *Food Chem*, 80, 255–260.

OLSEN K, KRISTENSEN D, RASMUSSEN J T and STIBSTED L H (2004), 'Comparison of the effect of high pressure and heat on the activity of bovine xanthine oxidase', *Milchwissenschaft*, 59, 411–413.

ORLIEN V, KNUDSEN J C, COLON M and SKIBSTED L H (2006), 'Dynamics of casein micelles in skim milk during and after high pressure treatment', *Food Chem*, 98, 513–521.

OSUMI M, YAMADA N, SATO M, KOBORI H, SHIMADA S and HAYASHI R (1992), 'Pressure effects on yeast cells ultrastructure: Change in the ultrastructure and cytoskeleton of the dimorphic yeast *Candida tropicalis*', in Balny C, Hayashi R, Heremans K and Masson P, *High Pressure and Biotechnology*, London, Libby Eurotext, 9–18.

PANDEY P K and RAMASWAMY H S (2004), 'Effect of high-pressure treatment of milk on lipase and γ-glutamyl transferase activity', *J Food Biochem*, 28, 449–462.

PATAZCA E, KOUTCHMA T and BALASUBRAMANIAN V M (2007), 'Quasi-adiabatic temperature increasing during high pressure processing of selected foods', *J Food Eng*, 80, 199–205.

PATTERSON M F and KILPATRICK D J (1998), 'The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry', *J Food Protect*, 61, 432–436.

PATTERSON M F, QUINN M, SIMPSON R and GILMOUR A (1995), 'Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods', *J Food Protect*, 58, 524–529.

PAYENS T A J and HEREMANS K (1969), 'Effect of pressure on the temperature-dependent association of β-casein', *Biopolymers*, 8, 335–345.

RADEMACHER B, PFEIFFER B and KESSLER H G (1998), 'Inactivation of microorganisms and enzymes in pressure-treated raw milk', in Isaacs N S, *High Pressure Food Science, Bioscience and Chemistry*, Cambridge, The Royal Society of Chemistry, 145–151.

RASANAYAGAM V, BALASUBRAMANIAN V M, TING E, SIZER C E, BUSH C and ANDERSON C (2003), 'Compression heating of selected fatty food materials during high-pressure processing', *J Food Sci*, 68, 254–259.

REDDY I M, KELLA N D K and KINSELLA J E (1988), 'Structural and conformational basis of the resistance of β-lactoglobulin to peptic and chymotryptic digestion', *J Agric Food Chem*, 36, 737–741.

REGNAULT S, THIEBAUD M, DUMAY E and CHEFTEL J C (2004), 'Pressurisation of raw skim milk and of a dispersion of phosphocaseinate at 9ºC or 20ºC: effects on casein micelle size distribution', *Int Dairy J*, 14, 55–68.

REGNAULT S, DUMAY E and CHEFTEL J C (2006), 'Pressurisation of raw skim milk and of a dispersion of phosphocaseinate at 9ºC or 20ºC: effects on the distribution of minerals and proteins between colloidal and soluble phases', *J Dairy Res*, 73, 91–100.

RITZ M, THOLOZAN J L, FEDERIGHI M and PILET M F (2002), 'Physiological damages of *Listeria monocytogenes* treated by high hydrostatic pressure', *Int J Food Microb*, 79, 47–53.

SCHRADER K and BUCHHEIM W (1998), 'High pressure effects on the colloidal calcium phosphate and the structural integrity of micellar casein in milk. II. Kinetics of the casein micelle disintegration and protein interactions in milk', *Kieler Milchw Forsch*, 50, 79–88.

SCOLLARD P G, BERESFORD T P, MURPHY P M and KELLY A L (2000a), 'Barostability of milk plasmin activity', *Lait*, 80, 609–619.

SCOLLARD P G, BERESFORD T P, NEEDS E C, MURPHY P M and KELLY A L (2000b), 'Plasmin activity, β-lactoglobulin denaturation and proteolysis in high pressure treated milk', *Int Dairy J*, 10, 835–841.

SIMPSON R K and GILMOUR A (1997), 'The effect of high hydrostatic pressure on *Listeria monocytogenes* in phosphate-buffered saline and model food systems', *J Appl Microbiol*, 83, 181–188.

SMIDDY M A, MARTIN J E G H, KELLY A L, DE KRUIF C G and HUPPERTZ, T. (2006), 'Stability of casein micelles cross-linked by transglutaminase', *J Dairy Sci*, 89, 1906–1914.

STEWART D I, KELLY A L, GUINEE T P and BERESFORD T P (2006), 'High pressure processing: review of application to cheese manufacture and ripening', *Austral J Dairy Technol*, 61, 170–178.

STYLES M F, HOOVER D G and FARKAS D F (1991), 'Response of *Listeria monocytogenes* and *Vibrio parahaemolyticus* to high hydrostatic pressure', *J Food Sci*, 56, 1404–1407.

SWAISGOOD H E (2003), 'Chemistry of caseins', in Fox P F and McSweeney P L H, *Advanced Dairy Chemistry, Volume 1: Proteins*, New York, Kluwer Academic/Plenum Publishers, 139–201.

TIMSON W J and SHORT A J (1965) 'Resistance of microorganisms to hydrostatic pressure', *Biotechnol Bioeng*, 7, 139–159.

VAN DEN BERG R W, HOOGLAND H, LELIEVELD H L M and VAN SCHEPDAEL L (2003), 'High pressure equipment designs for food processing applications', in Hendrickx M E G and Knorr D, *Ultra High Pressure Treatment of Foods*, New York, Kluwer Academic/Plenum Publishers, 297–313.

VAN OPSTAL I, VANMUYSEN S C M and MICHIELS C W (2003), 'High sucrose concentration protects *E. coli* against high pressure inactivation but not against high pressure sensitisation to the lactoperoxidase system', *Int J Food Microbiol*, 88, 1–9.

WALSTRA P, WOUTERS J T M and GEURTS T J (2006), *Dairy Science and Technology*, Boca Raton, FL, CRC Press.

WOUTERS P C, GLAASKER E and SMELT J P P M (1998), 'Effects of high pressure on inactivation kinetics and events related to proton efflux in *Lactobacillus plantarum*', *Appl Environ Microbiol*, 64, 509–514.

WROBLEWSKA B, KARAMAC M, AMAROWICZ R, SZYMKIEWICZ A, TROSZYNSKA A and KUBICKA E (2004), 'Immunoreactive properties of peptide fractions of cow whey milk proteins after enzymatic hydrolysis', *Int J Food Sci Technol*, 39, 839–850.

YE A, ANEMA S G and SINGH H (2004), 'High-pressure-induced interactions between milk fat globule membrane proteins and skim milk proteins in whole milk', *J Dairy Sci*, 87, 4013–4022.

ZEECE M, HUPPERTZ T and KELLY A L (2008), 'Effect of high-pressure treatment on *in-vitro* digestibility of β-lactoglobulin', *Innov Food Sci Emerg Technol*, 9, 62–69.

ZOBRIST M R, HUPPERTZ T, UNIACKE T, FOX P F and KELLY A L (2005), 'High pressure-induced changes in rennet-coagulation properties of bovine milk', *Int Dairy J*, 15, 655–662.

17

Pasteurization of milk with pulsed electric fields

G. Barbosa-Cánovas and D. Bermúdez-Aguirre, Washington State University, USA

Abstract: Pulsed electric fields (PEF) is a novel and very promising technology for pasteurization of pumpable foods. The food enters the PEF chamber, flows between two electrodes, and undergoes pulsing by electric fields. Microorganisms present in the food are inactivated as a result. There are theories regarding the mechanism of this inactivation. One of the most studied theories is related to electroporation of cell membranes. PEF treatment times are very short (microseconds), and processing temperatures are near room temperature or below, the main reason why PEF is called a nonthermal technology. Due to PEF's short processing times and low temperatures, foods keep their original sensorial and nutritional characteristics after processing. The effectiveness of PEF to inactivate bacteria and extend the shelf-life of food also ensures the microbiological quality of the final product. Energy savings using PEF are also important compared with conventional thermal treatment. This chapter presents the basic principles of PEF technology with special focus on its use in processing milk. Microbiological and enzymatic studies are mentioned as well. The advantages of this technology and current challenges and limitations are discussed throughout the chapter.

Key words: milk pasteurization, pulsed electric fields, microbial inactivation.

17.1   Introduction: key issues

Conventional methods for pasteurization of milk involve the use of heat, which ensures inactivation of pathogenic bacteria, reduces the number of spoilage microorganisms, and affects the activity of some native enzymes present in raw

milk. The main goal of pasteurization is to produce a safe milk product with extended shelf-life under refrigeration for consumers.

However, during the pasteurization process the use of extreme temperature conditions affects certain milk properties. Nutritional and sensorial characteristics are compromised at the time of microbial inactivation. Furthermore, despite the use of refrigerated conditions after pasteurization, milk has a limited storage life (three weeks or less) due to the heat-resistant spores of spoilage microorganisms, enzymatic activity, and the possible presence of other nonpathogenic microorganisms that survived pasteurization.

The conventional pasteurization method widely used around the world involves the use of heat (72ºC) and short processing times (15 s). After the process, milk is bottled and stored under refrigerated conditions until used by the consumer. There is a variation in milk processing that involves the use of high temperature (138ºC) with extremely short holding times (2 s); in this process all spoilage microorganisms are inactivated as well as most of the spores and enzymes. After the process, milk is packaged under aseptic conditions that allow storage for several months without refrigeration. However, the main disadvantage of this milk, known as UHT milk, is the undesirable change in taste and color, causing it to be rejected by consumers.

So, the pursuit of alternative pasteurization methods has become a priority for food scientists. Such methods must avoid thermal damage to milk properties while at the same time improve the microbial quality of the product. One alternative that has been effective in achieving these requirements is the use of pulsed electric fields (PEF), a novel technology that uses electric fields applied as pulses as a preservation factor. The advantages of PEF technology are microbial inactivation, reduction in enzyme activity, and minimal changes in milk characteristics. This chapter describes pulsed electric fields technology as a viable alternative for pasteurization of milk, beginning with the basic principles of the technology, followed by a description of the mechanisms of cell inactivation with examples showing the effects of PEF on pathogenic and spoilage bacteria, and enzyme inactivation. A brief evaluation of the characteristics of milk following PEF treatment and further research needs are also included.

17.2   Principles of the technology

Pulsed electric fields technology is the application of very short pulses (micro- to milliseconds), at an electric field intensity of 10–80 kV/cm, applied to a food product held between two electrodes inside a chamber, usually at room temperature. Food is capable of transferring electricity because of the presence of several ions, giving the product in question a certain degree of electrical conductivity. So, when an electrical field is applied, electrical current flows into the liquid food and is transferred to each point in the liquid because of the charged molecules present (Zhang *et al.*, 1995). The use of pulsed electric fields to pasteurize liquid food has high potential and a promising future in food

processing as a whole (Floury *et al.*, 2006a) because of the method's extremely short treatment times and its overall advantages compared with thermal pasteurization methods.

The use of electricity to pasteurize milk is not new. There are reports of different forms of electrical pasteurization used in the past, such as ohmic heating, microwave, low electric field stimulation, high voltage arc discharge, and low voltage alternating current (Barbosa-Cánovas *et al.*, 1999). In the early 1900s, there were also some reports on the use of electricity to sterilize milk. This sterilization process, known as Electro-pure, was able to inactivate pathogenic bacteria that normally show resistance to thermal treatment (Barbosa-Cánovas *et al.*, 1999); in 1961, Doevenspeck showed the feasibility of inactivating pathogenic bacteria using strong electric fields (up to 40 kV) and frequencies (up to 1000 Hz) (Bolado-Rodríguez *et al.*, 2000). In more recent years, the use of pulsed electric fields in processing milk has again been tested; in the late 1980s, Dunn and Pearlman studied the inactivation of *Salmonella* Dublin by PEF, and found that no cells were present after PEF treatment; the storage life of milk was also extended, due to the low number of spoilage bacteria cells present in the product (Barbosa-Cánovas *et al.*, 2000). From that point forward, many studies on microbial inactivation have been carried out using this nonthermal technology with favorable and enhanced results, most of them related to milk and milk products.

Currently, research of pulsed electric fields technology is ongoing around the world. Table 17.1 lists some of the institutions devoted to investigating the feasibility of this novel technology at an industrial level. Most of the research conducted up until now has been in the laboratory and on a pilot plant scale level, and has shown promising results. A number of institutions listed in Table 17.1 are now involved in the task of transferring and scaling up PEF technology to the industrial level for pasteurization of milk and other pumpable products. Because of the high potential of pulsed electric fields as a milk pasteurization alternative, in the next few years it can be foreseen that some of the most important dairy industries in the world will be using this emerging technology to process milk products.

The basis for this prediction is PEF's ability to inactivate microorganisms in milk, reduce enzymatic activity, and extend shelf-life with negligible changes in the quality of milk. Again, the reasons for this are quite simple: processing times are very short and processing temperature is low. To further demonstrate, a food product is held in place between two electrodes for a very short duration; the electrical discharge then administered is high enough to inactivate the microorganisms present in the food; this inactivation is due to change in cell membrane permeability, and is called electroporation or electropermeabilization. According to the intensity of the field strength, electroporation can be either reversible (cell membrane discharge) or irreversible (cell membrane breakdown or lysis), but this effect can be controlled depending on the application (Ho and Mittal, 1996). In specific cases, reversible electroporation is highly desirable, not only in bacteria, but also in some tissues in extracting

Table 17.1 Institutions around the world currently working with pulsed electric fields technology

Institution	Country
University of Buenos Aires	Argentina
Food Science Australia	Australia
Catholic University of Leuven	Belgium
University of Guelph	Canada
McGill University	Canada
University of Montpellier	France
University of Bordeaux	France
German Institute of Food Technology	Germany
Berlin University of Technology	Germany
KEKI	Hungary
Ohio State University	USA
Icetek	Iceland
University of Salerno	Italy
University of Auckland	New Zealand
TNO	Netherlands
Unilever Research Vlaardingen	Netherlands
Agrochemistry and Food Technology Institute (IATA)	Spain
University of Lleida	Spain
University of Zaragoza	Spain
SIK Göteborg	Sweden
University of Lund	Sweden
Nestlé	Switzerland
Campden and Chorleywood Food Research Association	UK
National Center for Food Safety and Technology	USA
United States Department of Agriculture (USDA)	USA
Washington State University	USA

cellular components or introducing new material (e.g., plasmid DNA). In pasteurization, irreversible electroporation is required to ensure microbial death and to maintain the safety of the product. This process not only affects microorganisms but also the cells of plant and animal tissues, allowing extraction and diffusion processes to take place, and changing the mass and heat transfer mechanisms in food (Angersbach *et al.*, 2000).

17.3   Pulsed electric fields processing equipment

A pulsed electric fields system has some main components that deliver the electrical discharge into the product. Some of these components are as follows: high voltage power source, capacitor bank, switch, treatment chamber, fluid pump, heat exchangers and probes for voltage, current, and temperature (Bolado-Rodríguez *et al.*, 2000). A basic PEF system is shown in Fig. 17.1. Here, a tank can be seen containing a raw product; the product flows through the pipe system to the PEF treatment chamber, but it can be optionally heated in the heat

Fig. 17.1   Basic configuration of a pulsed electric fields system.

exchanger before entering. After entering the chamber, the fluid receives treatment; the fluid is cooled in another heat exchanger, and then transferred to an aseptic filling system where it is bottled. The system can also recirculate the fluid to reprocess the food and increase the effectiveness of the treatment. In this case the first heat exchanger may be used to cool down the product to complete the first loop.

Processing conditions that can affect the microbial and enzymatic quality of milk during PEF treatment include electric field strength, duration and number of pulses, processing temperature, pulse frequency, treatment time, flow rate, pulse shape, and operation mode (batch or continuous).

The amount of energy (Q) delivered into the chamber during PEF processing can be calculated using the capacitance of the discharging capacitor C and the charging voltage V as follows:

$$Q = \left(\frac{R_{Ch}}{R_T}\right) \frac{CV^2}{2}$$

where R_{Ch} is the electrical resistance of the chamber and R_T the total electrical resistance of the system (Sepúlveda *et al.*, 2005a).

During PEF processing there can be an increase in temperature depending on the processing conditions and product treated. However, this increase is usually negligible due to very short processing times and the use of heat exchangers to keep the process at ambient or even low temperatures. The change in the temperature (ΔT) into the product can be calculated using the following equation:

$$\Delta T = \frac{fQ}{F\rho C_p}$$

where f is the pulse frequency, Q the amount of energy delivered into the chamber, F the flow rate of the product, and ρ and C_p the density and specific heat of the food, respectively (Sepúlveda *et al.*, 2005a). This equation is very useful in designing the process for a specific product, and keeps the product under nonthermal processing conditions and ensures its overall quality. This equation is also extremely important in designing specific processing conditions in the PEF circuit so as to avoid electrical breakdown of equipment during significant increases of temperature.

Currently, more companies are devoted to manufacturing and adjusting PEF systems according to food industry/research needs; in the past only two major companies were working on commercialization of these systems (Barbosa-Cánovas *et al.*, 2000). In the coming years, pulsed electric fields will be one of the main innovations adopted by the food industry, with the dairy sector being one of the main users.

In the following section the main components of the PEF system are discussed, providing a better understanding of how the process works. Some processing parameters are also described to explain how certain conditions could be enhanced to improve the performance of PEF equipment.

17.3.1   Treatment chamber

One of the key components in the PEF system is the treatment chamber, because this is where the electrical discharge is applied into the food and where microbial and enzymatic inactivation takes place. A common chamber consists of two or more electrodes held inside the chamber. There are different configurations of electrodes such as parallel plates and wires, concentric, rod-plate (Bolado-Rodríguez *et al.*, 2000), and coaxial. A schematic view of some of the most common treatment chambers used in food processing is shown in Fig. 17.2. Parallel plates are mainly used in batch systems; the food is held between two electrodes while the product receives an electric discharge. The coaxial and co-field chambers are used in continuous systems; in the coaxial chamber the food flows through the chamber through a gap between two electrodes. Generally the inner electrode is the high voltage electrode and the outer electrode is the one grounded. The last design shown in Fig. 17.2 represents a co-field treatment chamber, which is used in the latest PEF systems. This chamber consists of two hollow electrodes (anode and cathode) separated by an insulator with a tube through which the product flows. The design of the chamber should allow for a homogeneous electric field inside, thus exposing the food to homogeneous electric field intensity (Loeffler, 2006).

Currently, one of the main problems in PEF processing is the electrical breakdown of the system (i.e. the development of arcing). This situation is often observed in the processing of foods with bubbles while inside the chamber,

Fig. 17.2   Different PEF chamber configurations: (a) parallel plates, (b) coaxial and (c) co-field chambers.

because air does not conduct electricity. When the electrical discharge is applied, the contact between the air and the electric charge produces an electric arc or spark with subsequent explosion in the system. Bubbles are naturally present in some foods or can be formed during PEF processing if the temperature of the system is elevated, resulting in boiling of the food or evaporation of the liquid. Other possible reasons for arcing are related to the surface of the electrodes and the design of the chamber, which has been built to provide the food with a uniform electric field (Bolado-Rodríguez *et al.*, 2000). The PEF treatment can be delivered in batch or continuous mode according to the original setup of the equipment; however, most current systems and those in the process of being validated for industrial use are operated in continuous mode, which is the most efficient of the systems.

17.3.2   Pulse wave shape

The pulse wave shape used in PEF technology for food engineering applications can be either monopolar or bipolar. The monopolar form is classified as constant, rectangular, exponential or mixed, while bipolar wave shapes are sinusoidal, triangular, trapezoidal, continuous rectangular, discontinuous rectangular, or discontinuous exponential (Loeffler, 2006).

The pulse wave shape is delivered according to the configuration of the circuitry inside the PEF equipment; wave shape can also be changed in the same unit. Some of the most explored wave shapes for microbial inactivation are exponentially decaying, square-wave, bipolar, and oscillatory wave shapes (Barbosa-Cánovas *et al.*, 1999; Bolado-Rodríguez *et al.*, 2000). Some of the pulsed wave forms are presented in Fig. 17.3; the exponential pulse is characterized by a fast increase to maximum voltage, and a slow decrease to zero; during the square wave pulse the voltage is increased and decreased quickly, but in between, the voltage is held constant for a specific time. Bipolar forms are characterized by the presence of one positive and one negative pulse, displaying the same behavior as exponential or square wave shapes (Evrendilek and Zhang, 2005). The least effective pulse mode for microbial inactivation is the oscillatory one (Barbosa-Cánovas *et al.*, 1999). In general, square wave pulses are more energy efficient and lethal than exponential pulses; bipolar pulses are also more

Fig. 17.3   Different pulsed waveforms used in PEF processing.

lethal than monopolar pulses because of the stress generated in the cell membrane (Bolado-Rodríguez *et al.*, 2000).

17.4   Microbial inactivation

PEF is known as a nonthermal technology, because of the electric field strength delivered for short treatment times during application (microseconds to milliseconds) and its ability to reduce important bacterial loads without the use of heat. Important damage in the cell membrane, a result of the electric field, is one of the main reasons for microbial inactivation.

Pulsed electric fields treatment has the ability to inactivate bacteria in milk, as shown in Table 17.2. The bacteria shown to be inactivated in the different media include *Escherichia coli* (Evrendilek and Zhang, 2005), *Salmonella*, *Staphylococcus aureus* (Sobrino-López *et al.*, 2006; Evrendilek *et al.*, 2003), *Listeria monocytogenes* (Reina *et al.*, 1998), *Pseudomonas fluorescens* (Craven *et al.*, 2008), *Listeria innocua* (Calderón-Miranda *et al.*, 1999), *Lactobacillus brevis* (Sobrino-López and Martín-Belloso, 2008), and *Bacillus cereus* (Odriozola-Serrano *et al.*, 2006). Nevertheless, some microorganisms tested in milk, such as *Corynebacterium* spp. and *Xanthomonas maltophilia,* have been shown to be resistant to PEF inactivation (Odriozola-Serrano *et al.*, 2006). The processing conditions and the extent of inactivation for a number of these bacteria are further presented in Table 17.2.

The mechanism of cell inactivation with PEF use is known as electroporation, electrofusion or electropermeabilization. This mechanism of bacterial inactivation is based on the dielectric rupture theory (Bolado-Rodríguez *et al.*, 2000); it is also known as dielectric breakdown (Hülsheger *et al.*, 1981). Cell membranes are electrically insulated and their natural transmembrane potential (TMP) is around 60 to 110 mV. However, pores are formed when the natural TMP of the cell is exceeded (up to 1 V) with electric fields application, and when the electrical charge (buildup) accumulates on both sides of the membrane; as a result the permeability of the cell changes, reducing its viability (Heinz *et al.*, 2002; Grahl and Märkl, 1996; Ho and Mittal, 1996). Electroporation takes place in three main phases: pore formation when the electric field is applied; pore expansion; and finally, pore shrinkage and resealing (Ho and Mittal, 1996). Pores can be present in the lipid and protein parts of the cell

Table 17.2 Examples of microbial inactivation in milk using pulsed electric fields technology

Medium of treatment	Microorganisms	Processing conditions	Log inactivation	References
Milk ultrafiltrate	*Salmonella enteritidis*	55 kV/cm, 250 ns (pulse width), 20ºC (inlet temperature), maximum energy 80 kJ/kg	1.4	Floury *et al.* (2006a)
Skim milk	*Salmonella enteritidis*	47 kV/cm, 500 ns (pulse width), 62ºC (inlet temperature), 19 s	2.3	Floury *et al.* (2006b)
Skim milk	*Escherichia coli* O157:H7	24 kV/cm, 141 μs (total time), 2.8 μs pulse duration, bipolar and monopolar square wave	1.88 bipolar mode, 1.27 monopolar mode	Evrendilek and Zhang (2005)
UHT milk (whole, 1.5%, skim)	*Staphylococcus aureus*	35 kV/cm, 150 bipolar pulses (8 μs each)	4.5 (no significant effect on fat content)	Sobrino-López *et al.* (2006)
Sterile UHT milk	*Pseudomonas* strains	31 kV/cm, 55ºC, 19.6 μs (2 μs pulse width)	>5	Craven *et al.* (2008)
Raw skim milk	Enterobacteriaceae	25–37 kV/cm, 200 Hz, 2 μs, 15–60ºC	Not detected	Shamsi *et al.* (2008)
Fat-free milk	*Escherichia coli*	41 kV/cm, 63 pulses (2.5 μs), 37ºC	4.0	Dutreux *et al.* (2000)
Homogenized and pasteurized milk	*Salmonella* Dublin	36.7 kV/cm, 63ºC, 40 pulses, 100 μs	Not detected	Vega-Mercado *et al.* (1997)
Homogenized and pasteurized milk	*Escherichia coli* (ATCC-10536)	28.6 kV/cm, 42.8ºC, 23 pulses, 100 μs	3.0	Vega-Mercado *et al.* (1997)
Milk	*Lactobacillus brevis*	22 kV/cm, 45–50ºC, 20 pulses, 20 μs	4.6	Vega-Mercado *et al.* (1997)

Fig. 17.4 Mechanism of electroporation induced during pulsed electric fields processing: (a) normal cell; (b) stressed cell under action of electric field strength; (c) accumulation of electric charges at both sides of the membrane, and formation of some pores; (d) total destruction of cell membrane, irreversible pore formation, and release of intracellular material.

membrane; when the number of pores is too large compared to the size of the microorganism, the destruction of the cell wall is imminent, with subsequent death of the organism (Węsierska and Trziszka, 2007). According to the intensity of the treatment (electric field strength, number of pulses, temperature) the process of electroporation can be reversible or irreversible; thus, to pasteurize milk, the conditions used must be adequate to ensure the lethality of the cells because of the possibility of irreversible electroporation (Fig. 17.4). Reversible electroporation is used in some research areas of biotechnology for the specific purpose of opening the cell, injecting some specific components such as DNA to modify certain characteristics of the cell, and then closing the pore; other processes such as extraction or infusion of some metabolites also requires reversible electroporation conditions. Some mechanical properties of the cell membrane such as elasticity can have a special role in affecting the extent of sensitivity against the electric field (Sensoy *et al.*, 1997).

Because of the huge diversity of cells, their behavior upon application of PEF is quite different. Some of the biological factors that affect the intensity and lethality of the treatment are size and shape of the cell, and morphological and biochemical properties (Heinz *et al.*, 2002). Regarding the size of microorganisms, the value of the membrane potential induced by the electric field strength is lowered and the inactivation achieved is reduced. For example, in a detailed study using four target microorganisms under the same PEF processing conditions, *Saccharomyces cerevisiae* was the most sensitive microorganism because of its larger size compared with smaller cells like *Listeria innocua*, *Leuconostoc mesenteroides*, and *Escherichia coli* (Aronsson *et al.*, 2001). Cells in the exponential phase of growth (cells undergoing division) are more susceptible to inactivation by PEF than those in the lag and stationary growth phases (Hülsheger *et al.*, 1983; Barbosa-Cánovas *et al.*, 2000). Also, the sensitivity of microorganisms to PEF depends on their genre, for example Gram-positive are more resistant than Gram-negative, probably because of the presence of the cell wall; yeasts are more sensitive than bacteria (Hülsheger *et al.*, 1983) because of their size and also because of a thinner membrane. A

comparative study using PEF to inactivate three different types of cells under the same processing conditions showed that the most resistant was *Listeria innocua* (Gram-positive), followed by *Escherichia coli* (Gram-negative); the least resistant was the yeast *Saccharomyces cerevisiae* (Aronsson *et al.*, 2005), confirming the above theory.

Another factor contributing to the efficacy of PEF is the initial cell concentration. Donsì *et al.* (2007) showed that when the initial cell concentration of yeast in the product is low, the inactivation achieved is higher, which agrees with the results of Butz and Tauscher (2002). Other researchers (Barbosa-Cánovas *et al.*, 1999) have found that the inactivation produced by PEF is independent of the initial cell concentration. However, it is not possible to generalize for all microorganisms because, as mentioned before, inactivation depends greatly on other cell characteristics and factors.

The spores of some spore-forming microorganisms tested under PEF have been shown to be resistant to the process. However, spores of *Clostridium tyrobutyricum*, *Bacillus cereus, Bacillus nivea*, and *Bacillus subtilis* have not been inactivated significantly under selected PEF processes (up to 60 kV/cm) even when applied in addition to some moderate thermal treatment, including in the presence of lysozyme (Bendicho *et al.*, 2002a). A higher inactivation (3 log cycles) in skim milk was observed with *Bacillus cereus* spores using electric field strengths up to 40 kV/cm and more than 48 pulses (2.5 μs), combined with a concentration of nisin (10 to 50 IU/ml) and temperature of 65ºC (Bermúdez-Aguirre *et al.*, 2008a). However no evidence of mold spore inactivation under PEF technology has yet been reported (Heinz *et al.*, 2002).

The combination of temperature and electric field strength seems to enhance the bactericidal effect of PEF treatment (Craven *et al.*, 2008; Floury *et al.*, 2006a, 2006b; Hülsheger *et al.*, 1981). The synergistic effect between heat and electric field strength could be due to decrease in the critical potential of membrane electrical breakdown, which increases the sensitivity of micro-organisms (Floury *et al.*, 2006a; Bolado-Rodríguez *et al.*, 2000).

Some studies conducted with whole milk and *Listeria innocua* as the target microorganism tested for inactivation under PEF have shown more than 4 log reductions after less than 10 s of treatment. In these experiments, one to 31 pulses (2.5 μs each) at electric field strengths 30 to 40 kV/cm were applied in combination with an average temperature of 55ºC. Interestingly, when the temperature was raised to 63ºC at the highest voltage and only three pulses (2 μs each) were applied, microbial inactivation was similar (more than 4 log reduction); total energy in the process also was decreased from 244 J/ml to 44 J/ml using a thermal regeneration system (Sepúlveda, 2003). Here, it is clear that PEF technology offers the ability to enhance microbial inactivation through use of mild thermal treatments, but significant energy savings can be achieved as well when the heat generated during pulsing is used again in thermal regeneration.

Synergistic effects have been reported applying other preservation factors to inactivate microorganisms in milk, in addition to pulsed electric fields, for example (as mentioned) the use of natural antimicrobials such as nisin and

lysozyme (Sobrino-López and Martín-Belloso, 2006, 2008). Calderón-Miranda *et al.* (1999) showed the additive effect of combining nisin (10 to 100 IU nisin/ ml) with pulsed electric fields (30 to 50 kV/cm) in the treatment of skim milk to inactivate *Listeria innocua*. Other bacteriocins successfully used to enhance the microbial inactivation in combination with PEF are pediocin AcH, and pediocin AcH plus nisin; both were effective against *Listeria monocytogenes* Scott A, *Escherichia coli* O157:H7 932, and *Salmonella typhimurium* M1 (Kalchayanand *et al.*, 1994). The combination of PEF, bacteriocins, and mild thermal treatment (52ºC) has also been reported to enhance microbial inactivation. The smart combination of these three factors (at mild levels) successfully inactivated native flora in milk; nisin, lysozyme and lyso:Chrisin (1:3 lysozyme:nisin mix) were used as target bacteriocins (Smith *et al.*, 2002).

A single medium of treatment seems to enhance microbial inactivation. When buffer solutions or fruit juices are used during PEF processing, microbial inactivation is higher than with milk under the same conditions (Martín-Belloso *et al.*, 1997). When the medium of treatment is more complex (e.g. when proteins and fats are present), microbial inactivation becomes more difficult with PEF processing (Sobrino-López *et al.*, 2006; Sobrino-López and Martín-Belloso, 2008).

Some studies have shown that when the electrical conductivity of the medium is decreased the inactivation rate increases; this is a result of the short peak electric fields generated in the product. Thus, when the ionic strength of the food is modified, microbial cell inactivation is higher because of the additional electroporation effects and compression in the cell. Conductance in milk is generated due to the presence of charged species, such as salts, which are mainly chlorides, phosphates, citrates, carbonates, and bicarbonates of potassium, sodium, calcium and magnesium; however, this electrical property can be decreased as the fat content in milk is increased (Mabrook and Petty, 2003). The presence of some cations in the medium such as Ca^{2+} and Mg^{2+} has demonstrated some protective effect in cells against PEF treatment. It is assumed that this is due to interaction between the cations and the cell membrane (Hülsheger *et al.*, 1981, 1983). Also, change in the pH of the medium appears to be a hurdle in microbial inactivation, because as the pH of the product is reduced, the death rate of microorganisms is higher (Barbosa-Cánovas *et al.*, 2000).

Indeed, no general conclusions can be reached regarding the inactivation patterns of each microorganism, as many factors must be taken into consideration during the inactivation process, including the medium of treatment, kind of microorganism, and processing conditions.

17.5   Modeling microbial inactivation

Most of the emerging technologies that appear to be useful in microbial inactivation do not follow first-order kinetics, as illustrated in survival curve data for conventional thermal processing. Thus, it is incorrect to assume a linear

relationship between the survival ratio S (N/N_0) and dose of treatment. Therefore, because of the nonlinearity of the microbial death kinetics achieved using PEF, mathematical models have been proposed to describe the inactivation patterns of the survival curves. Some of these models are based on mathematical equations that describe the dependence of cell inactivation under selected electric field strengths. For example, one of the first models used for PEF technology, proposed by Hülsheger and Niemann, describes the dependence of the inactivation ratio (S) on the electric field intensity (E):

$$\ln(S) = -b_E(E - E_C)$$

where b_E is the regression coefficient, E the electric field, and E_C the critical electric field applied (Barbosa-Cánovas *et al.*, 2000). An alternative model describing cell inactivation in terms of treatment time was also proposed by Hülsheger:

$$S = \left(\frac{t}{t_C}\right)^{\frac{-(E - E_C)}{K}}$$

where t is the treatment time, t_C the critical treatment time, and K the kinetic constant. A small value of K means low sensitivity to PEF, whereas higher values of K indicate higher microbial sensitivity to treatment (Barbosa-Cánovas *et al.*, 2000).

Other inactivation models have been proposed to describe the survival curve of different microorganisms in selected treatment media under specific processing conditions; most are based on common mathematical equations adapted to specific conditions in PEF treatments (Gómez *et al.*, 2005a, 2005b; Álvarez *et al.*, 2003; Rodrigo *et al.*, 2003).

17.6   Enzyme inactivation

Although more than 10 years has been dedicated to the study of microbial inactivation by PEF, only a few researchers have considered enzyme inactivation, despite the stability of most foods being dependent on enzymatic activity. Some authors point out that more intense PEF treatments are needed to inactivate enzymes compared with the inactivation of microorganisms (Shamsi *et al.*, 2008). However, these studies are controversial; other research teams have not observed significant reductions in enzyme activity with PEF treatment under similar conditions (Bendicho *et al.*, 2003).

Some enzymatic studies reported on milk using PEF technology include the following enzymes: plasmin, protease, alkaline phosphatase, lactoperoxidase, and lipase (Van Loey *et al.*, 2002; Ho *et al.*, 1997). The different inactivation levels reported are related to the configuration of the PEF system and the source of enzymes, as well as factors related to the PEF process. In another study, Bendicho *et al.* (2003) showed that experimental results varied depending on the fat content of milk used (i.e. skim or whole).

The actual mechanism of enzyme inactivation is still unclear, but may involve the following: unfolding, denaturation, and breakdown of bonds and reactions within the protein structure resulting from action of the electric field strength (Shamsi *et al.*, 2008; Ho *et al.*, 1997). Some of the general conclusions concerning enzyme inactivation are related to use of batch systems, which appear to be more efficient for enzyme inactivation than continuous systems. Thus, it can be concluded that enzyme inactivation is different from the behavior of microbial inactivation in many respects (Bendicho *et al.*, 2002b).

17.7   Overall quality of milk

Only a few studies have been reported on the effects of pulsed electric fields on the physicochemical, nutritional, and sensorial characteristics of milk after processing. Some have reported changes in the pH of milk following PEF processing, while Floury *et al.* (2006a) did not find changes in this physico-chemical parameter (pH 6.72) after processing skim milk up to 55 kV/cm at room temperature. However, using extreme PEF conditions to pasteurize and inactivate spores in whole milk (40 kV/cm; 240 pulses, 2.5 μs each; 50ºC) changed the pH from 6.3 to 6.44 (Bermúdez-Aguirre *et al.*, 2008b).

A change in skim milk viscosity was also reported after PEF treatment (45 to 55 kV/cm, 2.1 to 3.5 μs), showing a decrease in this value, which is probably a result of change in the casein micelles, as a decrease in casein micelle diameter was also observed. This decrease in casein micelle size affects many of the characteristics of milk in addition to viscosity; the clotting time and other functional and textural characteristics are also modified after PEF processing (Floury *et al.*, 2006a). This also could be the reason for the change in the density of milk after PEF treatment; after processing at 40 kV/cm, 240 pulses (2.5 μs each) and 50ºC, the density was changed from 1029.8 to 1025.7 kg/cm^3 (Bermúdez-Aguirre *et al.*, 2008b).

Several studies have demonstrated there are no significant differences between thermally treated milk and PEF treated milk, although the latter product does appear to maintain the sensorial characteristics of the original better (Bendicho *et al.*, 2003; Rowan *et al.*, 2000; Reina *et al.*, 1998; Grahl and Märkl, 1996). Shelf-life of milk can also be extended using PEF, which is a highly energy-efficient process (Bolado-Rodríguez *et al.*, 2000), with low maintenance costs and simple operation procedures (Węsierska and Trziszka, 2007).

17.8   Shelf-life extension of milk

The goal of nonthermal technologies is not only to inactivate pathogenic bacteria and to retain the fresh-like characteristics of the product; a longer storage life is also highly desirable. If shelf-life of products can be extended considerably (even when stable at room temperature), the feasibility of shipping

and marketing food products on a wider scale becomes possible, an excellent alternative for food processors and the dairy industry to consider; longer shelf-life also could reduce economic losses when there is overproduction. As mentioned earlier in this chapter, some preliminary studies on PEF processing showed that the shelf-life of milk can be extended by reducing the growth rate of certain spoilage microorganisms during storage (Evrendilek *et al.*, 2001). Chocolate milk treated under PEF (35 kV/cm; 1.4 μs pulse width; 45 μs total time) and PEF plus thermal treatment (60ºC) showed important shelf-life extension of samples stored at temperatures of 22ºC and 37ºC. When chocolate milk was stored at 4ºC, following PEF or PEF plus heat treatment, no microbial growth was detected after 197 days of storage (Evrendilek *et al.*, 2001). Recent studies have shown that the shelf-life of milk thermally pasteurized previously, followed by PEF treatment, can extend the storage life of milk considerably. According to Otunola *et al.* (2008), milk-borne bacteria can affect the microbial inactivation of pathogenic microorganisms in milk treated under PEF. This research suggests the possible effect of competitive flora in milk during PEF inactivation, which could be a problem when shipping milk long distances. However, if the milk needs to be re-pasteurized as a result of such transport, the combination of high temperature short time (HTST) and PEF may be beneficial. When milk is pasteurized under HTST and immediately electrically pulsed, a shelf-life of up to 60 days can be obtained; when applied as a follow-up, 8 days after HTST pasteurization, PEF can lengthen the shelf-life of the product up to 80 days in cold storage (4ºC) (Sepúlveda *et al.*, 2005a).

17.9   Drawbacks and limitations

Processing with PEF technology does have some limitations, largely due to the presence of particles in various foods and the high viscosity of others, as well as some degradation in electrode material.

The main problem in non-homogeneous food is related to the presence of particles, which during processing do not receive the same treatment as the liquid product itself. This mainly applies to food emulsions. During PEF processing the operational constraints are related to the different electrical conductivities of particles and the liquid being treated; as a result they respond differently to the electrical discharge. In regions with high electrical conductivity, the field strength is reduced but in regions with low conductivity the electrical strength is increased (Lelieveld *et al.*, 2007). The study of time–temperature–electric field strength profiles of liquids with particles, and some emulsions, is a research area with high potential for PEF processing of some dairy products.

During the last several years there have been numerous modifications of the electrode, treatment chamber, and overall equipment to improve performance as well as to minimize some technical operational issues. One of the key elements in the PEF system is the electrode inside the chamber, which in some PEF

Table 17.3   A comparison of estimated element intake in the consumption of an 8 oz (227 g) PEF-treated meal and the published daily dietary intake of adult consumers

Elements	Estimated intake in 8 oz meal (μg/8 oz)	Published daily dietary intake (μg/day) (Reilly, 2002)
Ti	0.015	600
Pt	0.045	0.3
Fe	0.392	14,800 (female adult)
		8700 (male adult)
B	3.575	1500

Source: adapted from Zhang (2007).

designs can consist of more than one electrode per chamber. The electrode must be designed to be in direct contact with the food and the material should be inert (sanitary type – food grade) but with good conductivity to transfer the electricity. Usually, electrodes are made of stainless steel. However, the presence of metals in food because of the degradation of some parts, specifically from the electrode, as well as the electrochemistry of the process, must be considered during the PEF process (Master *et al.*, 2007).

Some of the aspects to be considered in the design of a PEF process are the design of electrodes, the treatment devices, as well as the pulse shapes; all of them together will reduce possible corrosion and release of metal particles into the food. According to Master *et al.* (2007), corrosion of electrodes happens because of the presence of direct current (DC) leakage into the system and low frequency alternating current (AC) voltages with the subsequent presence of ions of iron, nickel, chromium and manganese in the food. Some toxicological studies after pulsing food with pulsed electric fields have shown that the presence of some metals is below the allowed daily intake limits for human consumption (Table 17.3), which shows the potential of this technology to pasteurize some products without the leaching of metal particles.

Furthermore, the accumulation of metal particles in the product can be reduced during the process by using short enough pulses to inactivate bacteria and avoid the cumulative build-up of charges. The accumulation of build-up charges from proteins and living cells into the electrode can distort the intensity of the electric field, which can be avoided in high percentage with the use of bipolar pulses (Evrendilek and Zhang, 2005). Some of the recommended materials for electrodes are stainless steel, gold, platinum, metal oxides (iridium/ruthenium) (Góngora-Nieto *et al.*, 2002) or carbon (Master *et al.*, 2007). The use of electrodes made from metals with special metallurgical contents (Master *et al.*, 2007) or the use of special polymer coatings (highly conductive) like polyacetylene are some available options to minimize the problem of electrode corrosion during the pulsing of liquid food.

Concerns have been raised regarding the possible presence of toxic substances in food after PEF processing because of the interaction between

electricity and some molecules in the product. According to Master *et al.* (2007), the electrochemical changes are minimal after processing, and the presence of hydrogen and oxygen has been reported but at negligible levels. Comparing PEF with other electrical processes applied to food, the minimization of electrochemical reactions is brought about by the configuration of the equipment as well as the use of uniform field geometries (Master *et al.*, 2007).

The second constraint concerning PEF equipment is related to the formation of electric arcs during processing. The natural presence of small air bubbles in some products or the bubbles formed during processing because of stirring or high temperature inside the treatment chamber (boiling the product) can lead to the electrical breakdown of the system. To avoid this problem that may limit operation at the industrial level, some improvements and devices have been incorporated to ensure that before processing no air bubbles are present throughout the system and that electric arcing will be not produced. In addition the temperature of the product can be kept lower than the boiling point of the product. The use of pressure inside the system works well to dissolve any small bubbles in the food before processing.

17.10   Conclusions

The application of pulsed electric fields is a technology that has a promising future in the coming years. Every day more and more researchers are working to validate the technology, and manufacturers are working to scale up this technology so that it can be used commercially. Also, the dairy industry is considering more seriously the possibility of replacing the conventional technology for thermal pasteurization with some of the available state-of-the-art PEF equipment to improve the overall quality of milk, considerably extending its shelf-life and exploring new markets, not only for milk, but also for other milk-based products with a promising and environmental technology such as PEF.

17.11   References

ÁLVAREZ I, VIRTO R, RASO J and CONDÓN S (2003) 'Comparing predicting models for the *Escherichia coli* inactivation by pulsed electric fields', *Innov Food Sci Emerg*, 4(2): 195–202.

ANGERSBACH A, HEINZ V and KNORR D (2000) 'Effects of pulsed electric fields on cell membranes in real food systems', *Innov Food Sci Emerg*, 1: 135–149.

ARONSSON K, LINDGREN M, JOHANSSON BR and RÖNNER U (2001) 'Inactivation of microorganisms using pulsed electric fields: the influence of process parameters on *Escherichia coli*, *Listeria innocua*, *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae*', *Innov Food Sci Emerg*, 2: 41–54.

ARONSSON K, RÖNNER U and BORCH E (2005) 'Inactivation of *Escherichia coli*, *Listeria innocua* and *Saccharomyces cerevisiae* in relation to membrane permeabilization and subsequent leakage of intracellular compounds due to pulsed electric field processing', *Int J Food Microbiol*, 99: 19–32.

BARBOSA-CÁNOVAS GV, GÓNGORA-NIETO MM, POTHAKAMURY UR and SWANSON BG (1999) *Preservation of Foods with Pulsed Electric Fields*, San Diego, Academic Press.

BARBOSA-CÁNOVAS GV, PIERSON MD, ZHANG QH and SCHAFFNER DW (2000) 'Pulsed electric fields', *J Food Sci Supplement*, 65(s8): 65–79.

BENDICHO S, BARBOSA-CÁNOVAS GV and MARTÍN O (2002a) 'Milk processing by high intensity pulsed electric fields', *Trends Food Sci Tech*, 13, 195–204.

BENDICHO S, ESTELA C, GINER J, BARBOSA-CÁNOVAS GV and MARTÍN O (2002b) 'Effects of high intensity pulsed electric field and thermal treatments on a lipase from *Pseudomonas fluorescens*', *J Dairy Sci*, 85, 19–27.

BENDICHO S, BARBOSA-CÁNOVAS GV and MARTÍN O (2003) 'Reduction of protease activity in milk by continuous flow high-intensity pulsed electric field treatments', *J Dairy Sci*, 86: 697–703.

BERMÚDEZ-AGUIRRE D, DUNNE P and BARBOSA-CÁNOVAS GV (2008a) 'Inactivation of *Bacillus cereus* spores under pulsed electric fields, mild thermal treatment and nisin in skim and whole milk', *IFT Annual Meeting Book of Abstracts*, New Orleans.

BERMÚDEZ-AGUIRRE D, YAÑEZ J, DUNNE P, DAVIES NM and BARBOSA-CÁNOVAS GV (2008b) 'Electrodepositing of milk materials during pulsed electric fields processing', *IFT Annual Meeting Book of Abstracts*, New Orleans.

BOLADO-RODRÍGUEZ S, GÓNGORA-NIETO MM, POTHAKAMURY U, BARBOSA-CÁNOVAS GV and SWANSON BG (2000) 'A review of nonthermal technologies', in Lozano J, Añón MC, Parada E and Barbosa-Cánovas GV, *Trends in Food Engineering*, Lancaster, PA, Technomic Publishing.

BUTZ P and TAUSCHER B (2002) 'Emerging technologies: Chemical aspects', *Food Res Int*, 35: 279–284.

CALDERÓN-MIRANDA ML, BARBOSA-CÁNOVAS GV and SWANSON BG (1999) 'Inactivation of *Listeria innocua* in skim milk by pulsed electric fields and nisin', *Int J Food Micro*, 51: 19–30.

CRAVEN HM, SWIERGON P, NG S, MIDGELY J, VERSTEEG C, COVENTRY MJ and WAN J (2008) 'Evaluation of pulsed electric field and minimal heat treatments for inactivation of pseudomonas and enhancement of milk shelf-life', *Innov Food Sci Emerg*, 9: 211–216.

DONSÌ G, FERRARI G and PATARO G (2007) 'Inactivation kinetics of *Saccharomyces cerevisiae* by pulsed electric fields in a batch treatment chamber: The effect of electric field unevenness and initial cell concentration', *J Food Eng*, 78: 784–792.

DUTREUX N, NOTERMANS S, WIJTZES T, GÓNGORA-NIETO MM, BARBOSA-CÁNOVAS GV and SWANSON BG (2000) 'Pulsed electric fields inactivation of attached and free-living *Escherichia coli* and *Listeria innocua* under several conditions', *Int J Food Microbiol*, 54: 91–98.

EVRENDILEK GA and ZHANG QH (2005) 'Effects of pulse polarity and pulse delaying time on pulsed electric fields-induced pasteurization of *E. coli* O157:H7', *J Food Eng*, 68: 271–276.

EVRENDILEK GA, DANTZER WR, STREAKER CB, RATANATRIWONG P and ZHANG QH (2001) 'Shelf-life evaluations of liquid foods treated by pilot plant pulsed electric field system', *J Food Process Pres*, 25: 283–297.

EVRENDILEK GA, ZHANG QH and RICHTER ER (2003) 'Application of pulsed electric fields to skim milk inoculated with *Staphylococcus aureus*', *Biosyst Eng*, 87(2): 137–144.

FLOURY J, GROSSET N, LECONTE N, PASCO M, MADEC MN and JEANTET R (2006a) 'Continuous raw skim milk processing by pulsed electric field at non-lethal temperature: Effect on microbial inactivation and functional properties', *Lait*, 86: 43–57.

FLOURY J, GROSSET N, LESNE E and JEANTET R (2006b) 'Continuous processing of skim milk by a combination of pulsed electric fields and conventional heat treatments: Does a synergistic effect on microbial inactivation exist?', *Lait*, 86: 203–211.

GÓMEZ N, GARCÍA D, ÁLVAREZ I, CONDÓN S and RASO J (2005a) 'Modelling the inactivation of *Listeria monocytogenes* by pulsed electric fields in media of different pH', *Int J Food Microbiol*, 103(2): 199–206.

GÓMEZ N, GARCÍA D, ÁLVAREZ I, RASO J and CONDÓN S (2005b) 'A model describing the kinetics of inactivation of *Lactobacillus plantarum* in a buffer system of different pH and in orange and apple juice', *J Food Eng*, 70: 7–14.

GÓNGORA-NIETO MM, SEPÚLVEDA DR, PEDROW P, BARBOSA-CÁNOVAS GV and SWANSON BG (2002) 'Food processing by pulsed electric fields: Treatment delivery, inactivation level and regulatory aspects', *Lebensm-Wiss Technol*, 35: 375–388.

GRAHL T and MÄRKL H (1996) 'Killing of microorganisms by pulsed electric fields', *Appl Microbiol Biotechnol*, 45: 148–157.

HEINZ V, ÁLVAREZ I, ANGERSBACH A and KNORR D (2002) 'Preservation of liquid foods by high intensity pulsed electric fields – basic concepts for process design', *Trends Food Sci Tech*, 12: 103–111.

HO SY and MITTAL GS (1996) 'Electroporation of cell membranes: a review', *Crit Rev Biotechnol*, 16(4): 349–362.

HO SY, MITTAL GS and CROSS JD (1997) 'Effects of high field electric pulses on the activity of selected enzymes', *J Food Eng*, 31: 69–84.

HÜLSHEGER H, POTEL J and NIEMANN EG (1981) 'Killing of bacteria with electric pulses of high field strength', *Radiat Environ Biophys*, 20: 53–65.

HÜLSHEGER H, POTEL J and NIEMANN EG (1983) 'Electric field effects on bacteria and yeast cells', *Radiat Environ Biophys*, 22: 149–162.

KALCHAYANAND N, SIKES T, DUNNE CP and RAY B (1994) 'Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins', *Appl Environ Microbiol*, 60(11): 4174–4177.

LELIEVELD HLM, MASTWIJK HC and VAN DEN BOSCH HFM (2007) 'Pitfalls of pulsed electric fields processing', in Lelieveld HLM, Notermans S and Haan SWH, *Food Preservation by Pulsed Electric Fields, from Research to Application*, Cambridge, Woodhead Publishing.

LOEFFLER MJ (2006) 'Generation and application of high intensity pulsed electric fields', in Raso J and Heinz V, *Pulsed Electric Fields Technology for the Food Industry*, New York, Springer.

MABROOK MF and PETTY MC (2003) 'Effect of composition on the electrical conductance of milk', *J Food Eng*, 60: 321–325.

MARTÍN-BELLOSO O, QIN BL, CHANG FJ, BARBOSA-CÁNOVAS GV and SWANSON BG (1997) 'Inactivation of *Escherichia coli* in skim milk by high intensity pulsed electric fields', *J Food Proc Eng*, 20: 317–336.

MASTER AM, SCHUTEN HJ, MASTWIJK HC and LOMMEN A (2007) 'Toxicological aspects of preservation of food by pulsed electric fields', in Lelieveld HLM, Notermans S and de Haan SWH, *Food Preservation by Pulsed Electric Fields, from Research to Application*, Cambridge, Woodhead Publishing.

ODRIOZOLA-SERRANO I, BENDICHO-PORTA S and MARTÍN-BELLOSO O (2006) 'Comparative study on shelf life on whole milk processed by high intensity pulsed electric field or heat treatment', *J Dairy Sci*, 89: 905–911.

OTUNOLA A, EL-HAG A, JAYARAM S and ANDERSON WA (2008) 'Effectiveness of pulsed electric fields in controlling microbial growth in milk', *Int J Food Eng*, 4(7): 1–14.

REILLY C (2002) *Metal Contamination of Food*, Oxford, Blackwell Science.

REINA LD, JIN ZT, ZHANG QH and YOUSEF AE (1998) 'Inactivation of *Listeria monocytogenes* in milk by pulsed electric field', *J Food Protect*, 61(9): 1203–1206.

RODRIGO D, RUIZ P, BARBOSA-CÁNOVAS GV, MARTÍNEZ A and RODRIGO M (2003) 'Kinetic model for the inactivation of *Lactobacillus plantarum* by pulsed electric fields', *Int J Food Micro*, 81: 223–229.

ROWAN NJ, MACGREGOR SJ, ANDERSON JG, FOURACRE RA and FARISH O (2000) 'Pulsed electric field inactivation of diarrhoeagenic *Bacillus cereus* through irreversible electroporation', *Lett Appl Microbiol*, 31: 110–114.

SENSOY I, ZHANG QH and SASTRY SK (1997) 'Inactivation kinetics of *Salmonella* Dublin by pulsed electric field', *J Food Process Eng*, 20: 367–381.

SEPÚLVEDA DR (2003) *Preservation of fluid foods by pulsed electric fields in combination with mild thermal treatments*, PhD dissertation, Washington State University, Pullman, WA.

SEPÚLVEDA DR, GÓNGORA-NIETO MM, GUERRERO JA and BARBOSA-CÁNOVAS GV (2005a) 'Production of extended-shelf life milk by processing pasteurized milk with pulsed electric fields', *J Food Eng*, 67: 81–86.

SEPÚLVEDA DR, GÓNGORA-NIETO MM, SAN-MARTIN MF and BARBOSA-CÁNOVAS GV (2005b) 'Influence of treatment temperature on the inactivation of *Listeria innocua* by pulsed electric fields', *Lebensm Wiss Technol*, 38(2): 167–172.

SHAMSI K, VEERSTEG C, SHERKAT F and WAN J (2008) 'Alkaline phosphatase and microbial inactivation by pulsed electric field in bovine milk', *Innov Food Sci Emerg*, 9: 217–223.

SMITH K, MITTAL GS and GRIFFITHS MW (2002) 'Pasteurization of milk using pulsed electric field and antimicrobials', *J Food Sci*, 67(6): 2304–2308.

SOBRINO-LÓPEZ A and MARTÍN-BELLOSO O (2006) 'Enhancing inactivation of *Staphylococcus aureus* in skim milk by combining high-intensity pulsed electric fields and nisin', *J Food Protect*, 69(2): 345–353.

SOBRINO-LÓPEZ A and MARTÍN-BELLOSO O (2008) 'Enhancing the lethal effect of high-intensity pulsed electric field in milk by antimicrobial compounds as combined hurdles', *J Dairy Sci*, 91: 1759–1768.

SOBRINO-LÓPEZ A, RAYBAUDI-MASSILIA R and MARTÍN-BELLOSO O (2006) 'High-intensity pulsed electric field variables affecting *Staphyloccocus aureus* inoculated in milk', *J Dairy Sci*, 89: 3739–3748.

VAN LOEY A, VERACHTERT B and HENDRICKX M (2002) 'Effects of high electric field pulses on enzymes', *Trends Food Sci Tech*, 12: 94–102.

VEGA-MERCADO H, MARTÍN-BELLOSO O, QIN BL, CHANG FJ, GÓNGORA-NIETO MM, BARBOSA-CÁNOVAS GV and SWANSON BG (1997) 'Nonthermal food preservation: Pulsed electric fields', *Trends Food Sci Tech*, 8: 151–157.

WĘSIERSKA E and TRZISZKA T (2007) 'Evaluation of the use of pulsed electrical field as a factor with antimicrobial activity', *J Food Eng*, 78: 1320–1325.

ZHANG H (2007) *Pulsed Electric Field Processing*, Continue Education Program, Institute of Food Technologists, Chicago, IL.

ZHANG Q, BARBOSA-CÁNOVAS GV and SWANSON BG (1995) 'Engineering aspects of pulsed electric field pasteurization', *J Food Eng*, 25: 261–281.

18

Other novel milk preservation technologies: ultrasound, irradiation, microwave, radio frequency, ohmic heating, ultraviolet light and bacteriocins

G. Barbosa-Cánovas and D. Bermúdez-Aguirre, Washington State University, USA

Abstract: The search for processing alternatives to satisfy consumer requirements and to provide safe and healthy food is ongoing. Food scientists continue to study preservation factors in their exploration of new alternatives to current pasteurization and sterilization processes. A number of milk pasteurization alternatives have been studied more intensely than others in recent years with important and encouraging results. These include preservation factors other than heat (light, sound waves) and even heat generated inside the product (microwave). This chapter describes many of the novel thermal and nonthermal technologies available for pasteurization of milk, and the latest advances. Ultrasound, irradiation, and ultraviolet are among the nonthermal technologies discussed. Other novel thermal technologies such as microwave, ohmic heating, and radio frequency are mentioned in interesting detail. Most of these technologies show great potential in providing a pasteurized milk product with nutritional and sensorial characteristics similar to the original product, with shelf-life extended considerably. Aspects of these technologies related to equipment and processing conditions that need to be addressed to improve treatment, and represent current challenges for food scientists, are further presented in this chapter.

Key words: milk pasteurization, ultrasound, irradiation, microwave, radio frequency, ohmic heating, ultraviolet light.

18.1   Introduction

One of the main goals of conventional pasteurization is to inactivate any pathogenic microorganisms in the milk, while at the same time extending its shelf-life by reducing the microbial loads of spoilage bacteria as well as enzymatic activity. By combining time and temperature, the shelf-life of milk can be extended from 2 weeks at refrigerated conditions up to a couple of years if the packaging conditions and heat treatment are strong enough to achieve an almost 'sterile' environment. From a microbiological point of view the final product fulfills all legal requirements and is safe to drink by consumers. Furthermore, if the shelf-life is extended as a result of packaging conditions, the milk can be delivered to broader markets and safely consumed. However, the main disadvantage of commercial pasteurization is the degradation of the product's quality attributes and nutritional properties due to the intense heat treatment and subsequent destruction of proteins and vitamins, including undesirable changes to other characteristics such as flavor and color. To minimize such disadvantages in milk pasteurization, while at the same time ensuring the microbiological quality of the product, some new technologies based on different preservation factors have been tested as alternatives to traditional pasteurization and continue to be explored with successful and encouraging results.

18.2   Novel technologies for improving quality and their effectiveness

Nowadays, terminology such as 'novel processing' or 'emerging technologies in food science' has become quite common within the food science community. However, for consumers, such phrases are not commonly heard, nor is the terminology describing some food products as being processed by sound waves, microwaves, light or electricity. Nevertheless, the reality today is that these novel processing technologies are being tested for use in the food industry to improve the foods we eat, as they are capable of inactivating microorganisms, changing cell permeability, promoting chemical reactions, and even inactivating enzymes. Some novel technologies have been tested in model systems, for example oscillating magnetic fields; the technology was tested a long time ago but results were unfavorable, and further research has yet to be reported. On the other hand, ultrasound, radio frequency and ultraviolet are all under research in laboratories today in model and real food systems, while other novel technologies (e.g. cold plasma) are still undergoing initial testing. Irradiation, another and more accepted novel processing method, is a commercial reality in the processing of 240 food products in more than 50 countries; and nearing commercialization, a formal petition was submitted to the FDA in 2008 for the use of microwave technology. Clearly, all of these technologies are in different stages of development, but of significance here is that most have shown promising results in the pasteurization of milk, with only minor degradation in

Table 18.1 A summary of novel technologies undergoing research and approval for processing and preservation of foods

Technology[a]	Basic principle	Mechanism of inactivation	Status of technology	Common units related to the process
Bacteriocins (NT)	Similar to competitive flora	Pore formation, cell lysis, disruption of cell wall	Only nisin approved by FDA as GRAS[b]	IU/ml
Chemicals (NT)	Change in pH, production of free radicals and toxic compounds in cell	Cell disruption	Approved for use in food	ppm
Dense carbon dioxide (NT)	Moderate pressure, temperature, and carbon dioxide are combined	Cell wall disruption	Approved for juices	MPa, ºC
High hydrostatic pressure (NT)	Important increase in pressure with short treatment	Breakdown of cell wall, changes in cell morphology	Approved for food pasteurization and sterilization	MPa
Irradiation (NT)	Ionization, dissociation, and excitation	DNA damage	Used in more than 50 countries; 240 products	gray
Microwave (T)	Electrothermal process	Heating	Approved by FDA	ºC
Ohmic heating (T)	Electrical currents are passed though food	Heating	Approved for pasteurization of liquid eggs	ºC/s

Oscillating magnetic fields (NT)	Alignment to electric field strength	No cell inactivation	Unsuccessful research	tesla
Ozone (NT)	Aqueous solution or gaseous phase	Oxidative power, free radicals, cell membrane	Approved for water, other applications under research	ppm
Cold plasma (NT)	Electrical discharges	Changes in DNA, cell erosion, oxidation reactions	Under research	kV
Pulsed electric fields (NT)	Application of high intensity electric fields in microseconds	Electroporation of cells (reversible or irreversible)	Under research	kV/cm
Radio frequency (T)	Volumetric heating, friction between molecules	Heating	Under research	W, ºC/s
Ultrasound (NT)	Generation of sound waves in a liquid medium, producing thousands of bubbles (cavitation phenomenon)	Cell disruption, membrane permeability, breakdown of cells, generation of free radicals	Under research	μm
Ultraviolet (NT)	Exposure of product to UV radiant light	Mutation of DNA in cells	Approved for water and juices, under research for other products	J/m^2

[a] NT: nonthermal technology; T: thermal technology.
[b] GRAS: 'Generally Recognized As Safe'.

the quality and nutritional characteristics of the product. This chapter will describe the state-of-the-art of some of these technologies, as well as their basic principles, modes of action, and possible applications, including a detailed overview of recent advances in novel technologies applicable to processing and preservation of milk. Examples of novel processing technologies, the basic principles of each, and their mechanisms of cell inactivation are also presented in Table 18.1.

18.3   Ultrasound

Ultrasound is an emerging technology under research in the food engineering field for primarily liquid foods. There are two broad ranges of ultrasound application based on the level of frequency used: high frequency (2 to 10 MHz) and low frequency (20 to around 100 kHz), also called power ultrasound. The main difference between the two is the effect generated in the food. When high frequency ultrasound is applied to a food, a non-destructive effect is generated; there are different ultrasound parameters such as the attenuation coefficient that can relate important information regarding this effect, for example information about the product's structure or internal properties (McClements, 1995). However, with low frequency ultrasound application, the passage of sound waves through a liquid food causes the vibration of molecules, generating thousands of bubbles and producing physical effects in the food and microorganisms.

18.3.1   Definition
Power ultrasound (low frequency ultrasound) can cause physical disruption in food material, including cells, and also promotes chemical reactions in liquid foods. This kind of ultrasound is used in processes where the breakage of cells or other material is required, such as for the inactivation of microorganisms, the extraction of components from cells or tissues, or when a chemical reaction must be sped up or stopped, for example to accelerate or inactivate enzymatic activity in a food.

Power ultrasound is characterized by use of low frequencies, continuous mode of operation, and high power levels such as 10 and 10,000 W/cm^2 (Carcel *et al.*, 1998). The main effect of power ultrasound during passage of sound waves through the medium is called cavitation, which is the generation of thousands of bubbles. These bubbles have cycles of implosion and explosion that generate micro-currents and micro-storms; as the bubbles collapse, important increases in temperature and pressure result in the medium. The intensity of cavitation depends on the temperature, pressure, amplitude of the ultrasound wave, and media composition, among other factors. This part of the chapter will focus on how ultrasound technology can be applied to the dairy industry and its advantages and disadvantages.

18.3.2   Mode of action

The lethal effects of ultrasound in cells are attributed to the implosion and explosion of bubbles taking place during cavitation. According to the intensity of cavitation, the effect on cells can be lethal. Some studies on cells under thermo-sonication observed there were a number of physical effects in the cell membrane, mainly perforation, pitting, and surface granules; it was further observed that when the intensity of treatment increased there was breakdown of cells into their various parts (Bermúdez-Aguirre and Barbosa-Cánovas, 2008b). According to Earnshaw *et al.* (1995), the collapse of bubbles into the liquid affected the cells and removed particles from the cellular surface because of the 'hot spots' that formed instantly; pressure rose upward to 100 MPa and temperature to 5000 K in microseconds, but there was still enough time to disrupt and inactivate the microorganisms.

18.3.3   Milk processing

Pasteurization of milk is mainly needed to inactivate pathogenic bacteria, reduce spoilage microorganisms, and reduce enzymatic activity. This liquid food is a rich medium for bacterial growth; the proteins, fat, carbohydrates, minerals, vitamins and high percentage of water found in this product all make it an excellent substrate for the growth of bacteria, not only natural flora, but pathogenic bacteria in the environment and enzymes that thrive in this prosperous medium (Pelczar and Reid, 1972).

Several studies have been conducted with ultrasound to inactivate bacteria such as *Saccharomyces cerevisiae* (Tsukamoto *et al.*, 2004a, b; Guerrero *et al.*, 2005), *Escherichia coli* (Furuta *et al.*, 2004; Ananta *et al.*, 2005; Ugarte-Romero *et al.*, 2006), *Listeria monocytogenes* (Mañas *et al.*, 2000; Ugarte-Romero *et al.*, 2007), *Salmonella* (Cabeza *et al.*, 2004), and *Shigella* (Ugarte-Romero *et al.*, 2007) in different media, but only a few have been carried out in milk (Table 18.2). Microorganisms such as *Staphylococcus aureus*, *Bacillus subtilis* (Carcel *et al.*, 1998), *Salmonella* Typhimurium (Wrigley and Llorca, 1992), *Escherichia coli* (Zenker *et al.*, 2003), *Listeria monocytogenes* (D'Amico *et al.*, 2006; Pagán *et al.*, 1999; Earnshaw *et al.*, 1995), *Listeria innocua* (Bermúdez-Aguirre and Barbosa-Cánovas, 2008a), *Pseudomonas fluorescens* (Cameron *et al.*, 2009), and *Lactobacillus acidophilus* (Cameron *et al.*, 2008), including total aerobic microflora and coliforms (Villamiel *et al.*, 1999), have all been tested in milk under sonication.

The results of inactivation studies under sonication have been favorable, and show the positive effects of using sound waves to inactivate cells. In studies performed with *Listeria monocytogenes* in skim milk, the decimal reduction value was reduced from 2.1 min ($D_{60°C}$) (thermal treatment) to 0.3 min using ultrasound in combination with heat ($D_{60°C\&US}$) (Earnshaw *et al.*, 1995), a treatment known as thermo-sonication. When ultrasound was used in combination with pressure (mano-sonication), important reductions were also achieved in the inactivation of *Listeria monocytogenes* in skim milk. Using

Table 18.2 Examples of bacterial inactivation in milk and dairy products using novel technologies

Microorganism	Product	Technology	Condition/Result	References
Aerobes, coliforms, psychrotrophs	Milk	Microwave (MW)	6 log reduction using MW energy	NACMCF (2006)
Salmonella Typhimurium, *E. coli*, *Pseudomonas fluorescens*, *Streptococcus faecalis*	Milk	Microwave	MW at 78.6ºC (65 s) did not inactivate all microorganisms	NACMCF (2006)
Listeria monocytogenes	Milk	Microwave	MW at 71.1ºC (10 min) completely inactivated cells (8–9 log reductions)	NACMCF (2006)
Enterobacter sakazakii	Rehydrated infant milk formula, dried infant milk formula	Irradiation	1 kGy: 4 log reductions 9 kGy: 7 log reductions	Osaili *et al.* (2007)
Streptococcus thermophilus	Skim milk	Ohmic heating	70ºC/30 min, $D = 6.59$ min 75ºC/15 min, $D = 3.09$ min 80ºC/1 min, $D = 0.16$ min	Sun *et al.* (2008)
Escherichia coli	Skim milk	Ultraviolet	253.7 nm, 15ºC, 3600 rpm, 1.5 l/min: 3.24 log reductions	Milly *et al.* (2007)
Escherichia coli K-12	Milk	Radiofrequency	2 kW, 27.12 MHz, 1200 W, 55.5 s: 7 log reductions	Awuah *et al.* (2005)
Listeria innocua	Fat-free milk	Ultrasound	24 kHz, 100%, 63ºC, 10 min: 5 log reductions	Bermúdez-Aguirre and Barbosa-Cánovas (2008a)
Listeria monocytogenes	UHT milk	Ultrasound	Heat at 60ºC, $D_{60} = 2.1$ min Heat at 60ºC with sonication at 20 kHz, $D_{60\&US} = 0.3$ min	Earnshaw *et al.* (1995)
Escherichia coli K12 DH5α	UHT milk (pH 6.7)	Ultrasound	Heat at 60ºC, $D60 = 77$ s Ultrasound (110 μm) assisted with temperature (60ºC), $D_{60\&S} = 23$ s	Zenker *et al.* (2003)

ambient pressure and temperature, the decimal reduction value was 4.3 min in this study during ultrasound treatment; increasing the pressure up to 200 kPa reduced the D value to 1.5 min; using twice the previous pressure (400 kPa) reduced the D value to 1.0 min. When temperature was increased above 50ºC, the lethality of ultrasound on *Listeria* cells was enhanced (Pagán *et al.*, 1999).

Zenker *et al.* (2003) studied the inactivation of *Escherichia coli* K12DH5α in UHT milk using thermal treatment (60ºC); combining the same thermal treatment with ultrasound reduced the D value from 77 s to 23 s. These are examples of the additive effect of ultrasound combined with heat, leading to microbial inactivation.

Studies on UHT milk with a surrogate of *Listeria monocytogenes*, *Listeria innocua* ATCC 51742, have shown that inactivation of cells is possible with thermo-sonication treatment. After 30 min of treatment, almost 5 log reductions were achieved in fat-free milk, whereas up to 2.5 log reductions were achieved in whole milk. Intermediate inactivation levels in two milks (1% and 2% butterfat) were achieved (Bermúdez-Aguirre and Barbosa-Cánovas, 2008a). The efficiency of ultrasonication in milk processing for bacterial inactivation depends on the microbial strain, the medium, the size of cells, and the electrical power supplied, according to Cameron *et al.* (2009).

Thermo-sonication has also been useful in extending the shelf-life of raw whole milk (Bermúdez-Aguirre *et al.*, 2009) and UHT milk (Bermúdez-Aguirre and Barbosa-Cánovas, 2008a), by retarding the growth of mesophilic bacteria. In the first case, after the raw and whole milk were pasteurized at 63ºC and 120 μm of ultrasound for 30 min, mesophilic growth was lower than 2 log after 16 days at 4ºC. In the second case, after the UHT milk was treated with thermo-sonication under the same processing conditions, the growth of mesophilic bacteria was retarded compared with the control samples, regardless of storage temperature (ambient or refrigeration); however, the butterfat content was an important factor favoring the growth of bacteria. Studies in the use of sonication in combination with other novel technologies have been recently reported by Noci *et al.* (2009). Inactivation of *Listeria innocua* in low fat UHT milk was analyzed using only sonication and other hurdles such as temperature and pulsed electric fields (PEF). Results showed that thermo-sonication of milk with the addition of PEF treatment could enhance the inactivation of *Listeria innocua* in milk significantly.

Nutritional properties

Emerging technologies are under research to minimize thermal damage and to improve the quality of the final product, nutritional properties being one such area of focus. However, in addition to the goal of minimal changes in nutritional properties, these new technologies offer several advantages that are not limited to the final product, but also promote new ingredients for use in additional processed food products.

Some changes have been reported in the protein content of milk after sonication. Cameron *et al.* (2009) reported that after sonication of milk (750 W; 20 kHz; 124 μm) for up to 15 min at room temperature the protein content was

increased from 3.03 to 3.24% in raw milk; whereas in UHT milk (3.4% fat content) after treatment, protein content decreased slightly from 3.12 to 3.11%. The authors did not explain this behavior in milk. In comparison, studies conducted on raw milk after sonication (400 W; 24 kHz; 120 μm) at 63ºC showed a decrease in protein content ranging from 3.28 to 3.00%, depending on the intensity of the treatment (Bermúdez-Aguirre et al., 2009); with different butterfat content in UHT milk (fat-free, 1%, 2%, 3.4%), the protein decrease in samples was not significant compared with untreated sample, after similar processing conditions (Bermúdez-Aguirre and Barbosa-Cánovas, 2008a).

On the other hand, there was an increase in antioxidant activity in skim milk when subjected to sonication, an effect probably due to the action of cavitation in the disruption of the quaternary and tertiary structure of the proteins (Villamiel and de Jong, 2000). Some studies performed with thermo-sonication of raw whole milk have shown a significant difference ($p < 0.05$) between the crude protein of thermal-treated (63ºC) and thermo-sonicated (63ºC plus ultra-sound) samples compared with untreated milk. A synergistic effect was reported in milk treated with heat and ultrasound together in the inactivation of enzymes in a study of the potential denaturation of proteins (alkaline phosphatase, γ-glutamyltranspeptidase, lactoperoxidase, α-lactalbumin, and β-lactoglobulin) at 61, 70 and 75.5ºC. However, under these conditions, casein content did not change significantly (Villamiel and de Jong, 2000). It appears that the use of higher temperatures (above 60ºC) in combination with ultrasound is responsible for denaturation in proteins, whereas in a contrasting study, when skim milk was thermo-sonicated at temperatures below 50ºC, the soluble protein content was no different from the control (Wrigley and Llorca, 1992). Additionally, Cameron et al. (2009) reported that alkaline phosphatase was not inactivated using ultrasound at room temperature; however, lactoperoxidase activity was reduced after 5 or 10 min of sonication, but it was not totally inactivated.

One of the keys to processing milk under thermo-sonication is to find the ideal conditions supportive of microbial inactivation, while at the same preventing the denaturation of proteins. Consequently, more studies with a focus on protein denaturation must be performed to improve this technology.

Studies on butterfat content in milk after sonication have been conducted as well, showing one common result, the increase of butterfat content in milk (Bermúdez-Aguirre et al., 2009; Cameron et al., 2009). The result can be attributed to the disruption of the membranes of the milk fat globules, in releasing triacylglycerols into the medium; their release makes quantification with analytical techniques easier (Bermúdez-Aguirre et al., 2008); quantification with infrared light based techniques (Cameron et al., 2009) is also easier because of the size reduction in the fat globule's showing a bigger surface area.

18.3.4   Regulatory issues

Although ultrasound is under research for microbial inactivation in laboratories around world, a complete chemical profile of milk documenting the effects of

sonication after pasteurization is still needed to ensure that only non-toxic compounds are generated in milk with this technology. There is no evidence of any legal procedures that could potentially approve the use of ultrasound for milk processing and preservation at this time. It is possible that after more research has been conducted on more microorganisms in different media, including study of nutrient content, chemical profiles, and validation of results, the approval of this technology for commercial use could be a reality in the next few years.

18.3.5 Advantages and limitations
The advantages ultrasound offers the food industry and consumers compared with conventional pasteurization are many. While the inactivation of pathogenic bacteria can be achieved via shorter processing times, it is also important to note the significant improvements in the final quality of milk after processing. For example, the color of milk is whiter, which has been attributed to the change in size and microstructure of fat globules after sonication, providing the milk with a higher degree of homogenization and better (i.e. whiter) appearance (Bermúdez-Aguirre *et al.*, 2008). Minimal changes in protein and vitamin content have been reported in the few studies conducted in this area. To further verify the added advantages of ultrasound in processing and preserving milk, more detailed studies are needed on this novel technology.

18.4 Irradiation

Food irradiation is not a new technology; important data describing this technology date from the late nineteenth century (Molins, 2001). The idea of using irradiation to destroy microorganisms in food was first reported in conjunction with the discovery of radioactivity by Henri Becquerel in 1895 (Satin, 1996). Once irradiation began to be used to process foods, its main application was to improve the quality of several products. However, in the 1980s more regulations were approved by the United States Department of Agriculture (USDA), US Food and Drug Administration (FDA), World Health Organization (WHO), and European Community for different products and purposes (Molins, 2001). In 1980 the Food and Agriculture Organization (FAO), International Atomic Energy Agency (IAEA), and WHO stated that irradiation of food (average dose 10 kGy) does not represent a toxicological, micro-biological or nutritional risk for human beings (Satin, 1996; Morehouse and Komolprasert, 2004). Consequently, since the 1990s, more than 40 countries worldwide now use irradiation for various purposes in processing different foods (Molins, 2001).

18.4.1 Definition
Irradiation is related to the propagation of energy from the electromagnetic spectrum. Radio and television waves, microwaves, and ultraviolet and gamma

Fig. 18.1   The electromagnetic spectrum.

rays are some of the examples of radiation included in the spectrum (Fig. 18.1). Controversy among people in general is associated with the terminology 'food irradiation' and its correlation with 'nuclear radiation'; similarly, in France 'irradiation' is associated with 'nuclear radioactivity', and thus the term used there is ionization (Satin, 1996). Food irradiation is incorrectly used to describe the process in which ionizing radiation is applied to food. Recently, in the United States, the term used most frequently has been 'electronic pasteurization', a process that attempts to inactivate bacteria with irradiation (Molins, 2001).

18.4.2   Mode of action

Ehlermann (2002a) defines irradiation as a type of energy (enough to cause ionization) that is transferred to the food. There are three sources of ionizing radiation used to process food: gamma rays, X-rays, and electron beams (Satin, 1996). To produce gamma rays two radionuclides are needed: cobalt-60 (^{60}Co) and cesium-137 (^{137}Cs). Cobalt-60 produces gamma rays of 1.17 and 1.33 MeV; cesium-137 produces gamma rays of 0.66 MeV. Electron beams are generated by means of a linear accelerator, and energy levels are up to 10 MeV.

During irradiation of food with ionizing radiation, a cascade of secondary electrons with enough kinetic energy generates ionization of atoms and molecules and formation of free radicals. In foods with high moisture content, chemical species are formed from the radiolysis of water (Ehlermann, 2002a). The three main effects of irradiation of foods in general are ionization, dissociation, and excitation. When energy passes through the food, the product absorbs the energy, which then generates the desirable or undesirable changes in the irradiation process (Patil, 2004).

Irradiation can be used for sprout inhibition, ripening delay, insect disinfestation, inactivation of pathogens, and reduction of other microorganisms. The main mechanism of inactivation in irradiation is damage to the DNA in the cells (Ehlermann, 2002a). Depending on the purpose, the required dose varies from very low (e.g. 1 kGy) to higher than 10 kGy. Depending on the absorbed dose, irradiation can be classified as one of three processes: radicidation, radurization, and radappertization. In radicidation the goal is to reduce the number of non-spore-forming pathogenic bacteria and parasites. The low doses (lower than 0.4 kGy) used in this treatment are equivalent to irradiation pasteurization. The second process, radurization, is used specifically to improve shelf-life and the quality characteristics of a product. Doses are around 0.4 to 10 kGy. The third process, radappertization, is used to reduce the number and/or activity of microorganisms to a very low number, one that is not detectable by conventional methods. Doses are about 10 to 50 kGy, which is equivalent to irradiation sterilization (Barbosa-Cánovas *et al.*, 1998).

18.4.3   Milk processing

Irradiation can be used to inactivate pathogenic microorganisms in milk and products of milk (Osaili *et al.*, 2007). A number of microorganisms have been successfully inactivated with this technology: *Salmonella* and *Listeria* with doses lower than 10 kGy, and *Escherichia coli* O157:H7 with medium doses of irradiation, including *Staphylococcus aureus* cells, although toxins are not destroyed through irradiation (Crawford and Ruff, 1996).

In a study conducted on three types of milk (cow, buffalo and goat), inactivation of bacterial and spore-forming microorganisms was observed after combining gamma-irradiation and thermal treatment (70ºC for 15 s); meanwhile yeasts, molds and coliforms were totally inactivated under the same treatment (Naghmoush *et al.*, 1983). There is no important difference in the resistance of Gram-positive or Gram-negative bacteria and yeasts to inactivation under irradiation, although as before, spores are resistant to this technology (Jandal, 1990).

Enterobacter sakazakii, which is considered an opportunistic pathogen, has been associated with foodborne outbreaks in baby food products, especially in powdered infant milk formula (Osaili *et al.*, 2008). This microorganism has been inactivated in infant milk formula using irradiation at doses of 1 to 9 kGy, depending on the conditions of the sample; using the highest dose for dried products, up to 7 log reductions were achieved (Osaili *et al.*, 2007). Ionizing radiation seems to be more effective when applied soon after the manufacture of powdered infant formula (Osaili *et al.*, 2008). This use has potential in the milk industry because of the presence of this emerging pathogenic microorganism in baby foods.

Overall quality

The main chemical changes associated with irradiation of food result from the radiolysis of water with subsequent formation of free radicals and recombination

with other chemical compounds in the product; similar effects have been obtained with other conventional food processing technologies (Chawla *et al.*, 2009; Kaddouri *et al.*, 2008). Irradiation can change some of the chemical and physical properties of milk, a result of denaturation, degradation, polymerization, and rearrangement of proteins, vitamins and carotenoids, as well as hydroperoxide production (Jandal, 1990). The effects of gamma-irradiation on the proteins in cow's milk have been studied, applied at doses of 3, 5 and 10 kGy, at room temperature, and in the presence of air. Results showed that solubility of proteins was reduced after irradiation treatment, which could be a result of crosslinking of protein chains; however, *beta*-lactoglobulin antigenicity was not reduced by the treatment (Kaddouri *et al.*, 2008). Similar studies conducted on whey proteins treated under gamma-irradiation (0 to 100 kGy) showed the formation of crosslinked proteins after treatment, but also the formation of antioxidant compounds in glucose/amino acid solutions (Chawla *et al.*, 2009).

In a study using gamma-irradiation in combination with thermal treatment (70°C for 15 s) to pasteurize milk, the decrease in vitamin A and carotene content was observed as irradiation dose was increased (Naghmoush *et al.*, 1983). The intelligent combination of dose, temperature, and other processing conditions can reduce the negative effects in milk and milk-related products considerably.

Micronutrients such as minerals are not altered in a significant way in irradiated food. In fact, in some products minerals (e.g. phosphorus) are more available after irradiation. Losses due to irradiation are similar to those lost in other common food processing techniques (Urbain, 1986).

However, depending on the irradiation dose, some products can develop undesirable characteristics of flavor, color, and taste. Application of irradiation and thermal treatment can affect the flavor, ranging from slightly caramelized to strongly oxidized, whereas the color becomes whiter after processing (Naghmoush *et al.*, 1983). High doses (around 45 kGy) applied to milk under refrigerated conditions can produce browning and caramelized flavor, while ambient temperature can produce gelation. Using freezing temperatures in combination with irradiation can reduce the above problems, although a bitter flavor can be detected. Using lower doses of irradiation (20 kGy) in combination with vacuum and irradiation can be effective in processing milk without important changes in sensorial characteristics (Urbain, 1986).

18.4.4 Regulatory issues

At present there is no worldwide regulation pertaining to irradiation, although countries that have accepted irradiation as a food processing technology have established their own regulations. Regulations concerning the licensing of food plants, waste management, and environmental security are similar among the countries, but there is no one standard among them. The FDA views irradiation more as a food additive than a technology, and considers the source unimportant, whether it is from radioactive isotopes, particle accelerators, or X-rays. National

and international research in food irradiation is reviewed and evaluated by the IAEA, WHO, and FAO (Morehouse and Komolprasert, 2004). The list of countries that have approved food irradiation is large and more than 50 use irradiation to process foods today, for example Belgium, Bulgaria, Denmark, France, Germany, Spain, and the United Kingdom; the United States, Mexico, Canada, Chile, and Argentina; China, Japan, India, Indonesia, Thailand, and Korea; and South Africa and Ivory Coast (Barbosa-Cánovas *et al.*, 1998).

18.4.5   Advantages and limitations

Food irradiation appears to be an economically viable technology. Uses of this technology include reducing post-harvest losses, extending product shelf-life, inactivating foodborne pathogens, and more (Ashraf-Chaudry *et al.*, 2004). The features of this technology can further be listed as follows: (1) preserves food, (2) eliminates bacteria, molds, yeasts and insects, (3) produces non-toxic residues, (4) does not affect nutritional and sensorial quality, (5) changes some chemical properties, improving food quality, and (6) can be applied after product is packaged (Barbosa-Cánovas *et al.*, 1998). Finally, irradiation reduces the risk of foodborne illnesses, even in patients with compromised immune systems, and also in the case of astronauts traveling in space (Moy, 2005).

Some disadvantages of the technology are the unpleasant flavor that milk can acquire after treatment, and the resistance of spore-forming bacteria to the highest approved doses. Some limitations of irradiation could be reduced with the use of hurdle technology (Barbosa-Cánovas *et al.*, 1998). The main disadvantage of using irradiation as an additive in food processing or a food processing technology is the concern of consumers regarding its safety and the perceived issues related to cancer and radioactivity.

18.5   Microwave

Microwave can be classified as an electrothermal process for pasteurization of food; it is capable of inactivating some pathogenic microorganisms such as *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes*. One of the main disadvantages of using microwave energy to pasteurize food is the unevenness of temperature distribution throughout the process (NACMCF, 2006). However, much research is being conducted on this technology to fine-tune its effectiveness, which could result in it becoming a viable option for pasteurization and sterilization of foods in the near future. In the following section, a description of the technology and its particular use in milk pasteurization will be discussed.

18.5.1   Definition

Microwave (MW) is a form of electromagnetic wave energy that has been used successfully in the industry. Radiation at frequencies ranging from 300 MHz to

300 GHz corresponds with microwave energy in the electromagnetic wave spectrum. Lower frequencies corresponding with infrared and ultraviolet, and higher, are used for radio frequency (Sanga *et al.*, 2000; Piotrowski *et al.*, 2004). This range of frequencies is shown in Fig. 18.1.

Microwave frequencies are close to the frequencies found in radio, television, and radar waves; as a result, each country has its own specifications and is assigned a permitted frequency for microwave commercial use to avoid interference with communications. In North America there are two approved microwave frequencies, 915 and 2450 MHz (Sanga *et al.*, 2000); these frequency bands are called ISM (Industrial, Scientific, Medical) because they are set apart from telecommunications for use in industrial, scientific and medical applications (Regier and Schubert, 2001). The International Telecommunication Union (ITU) limits the number of frequency bands. The bands currently designated are:

- 2450 MHz in domestic ovens and industry
- 970 MHz, 915 MHz and 897 MHz in some countries for industrial applications
- 22,125 MHz, reserved for future use (Ehlermann, 2002b).

The frequency (*f*) is related to velocity of light (*c*) and wavelength (λ) such that:

$$f = \frac{c}{\lambda}$$

(Regier and Schubert, 2001).

18.5.2   Mode of action

Equipment for industrial applications has three main components: (1) microwave generator, (2) waveguide and applicator, and (3) other devices commonly used (e.g. transformer, rectifier, and instrument to control the energy supply) (Sanga *et al.*, 2000).

A high-frequency generator guides the waves into the oven and prevents the waves from leaving the chamber. Penetration in food depends on the wavelength, depth of material, type of product (composition, structure, dielectric properties, and geometry), and temperature (Okos *et al.*, 1992; Sanga *et al.*, 2000; Drouzas *et al.*, 1999; Hayta *et al.*, 2002). This kind of energy can be absorbed by materials containing water or other 'lossy' substances, such as carbon and other organics, and can be converted to heat (Maskan, 2000). When energy enters the food, molecular dipoles try to align the electric field orientation; they start to oscillate and generate heat in the food, leading to a drying process (Okos *et al.*, 1992; Ramaswamy *et al.*, 2001). The mechanisms under energy that are dissipated in the frequency range of the microwave system are free water polarization (γ dispersion), bound water polarization (δ dispersion), Maxwell–Wagner polarization (β dispersion), and ionic conductivity (Feng, 2000).

Because of the nature of the process, the main mechanism of cell inactivation using microwave energy is heat; thus, the effect on microorganisms is similar to the effect observed for cell inactivation using conventional thermal processing, i.e. denaturation of proteins and cell wall.

Dielectric properties

Atoms and molecules are present in common matter. Some are charged negatively (electrons) and others are charged positively (protons). When an electric field is applied, negative charges are oriented toward the positive electrode and positive charges are oriented toward the negative electrode. When this occurs in a dielectric material, it searches for the equilibrium state. Therefore, both effects are equal and opposite in the direction of the field applied. The redistribution of charges is called dielectric polarization (Kent, 2001). Dielectric properties, which are responsible for the success of microwave processes, are the dielectric constant (ϵ') and the dielectric loss factor (ϵ''); both are related to the electric permittivity complex ($\epsilon = \epsilon' - j\epsilon''$), where j is $\sqrt{-1}$. Another important parameter in microwave theory is the loss tangent expressed as $\tan \delta = \epsilon''/\epsilon'$ (Komarov *et al.*, 2004).

The dielectric constant (ϵ') is related to the distribution of the magnetic field in the material; it shows how efficiently the energy can be stored in the material or how well the material can polarize, in other words, how much heat can be produced in the material when exposed to microwave radiation. The loss factor (ϵ'') represents the loss interactions, which measures how the energy is dissipated into the material. The loss tangent (dissipation factor) is used to indicate the energy lost when passing through or being absorbed by the material (Sanga *et al.*, 2000; Kent, 2001; Feng, 2000). The ϵ' and ϵ'' depend on the frequency for a typical polar dielectric (Kent, 2001). Any water present in food has the highest loss factor by far, and because of this, heat is transmitted into the food during the microwave process (King, 1973). Water is a typical polar molecule, with two hydrogen atoms and one oxygen atom. The strength of the dipole is exceptional and the dielectric dispersion is of a large magnitude (Kent, 2001). For food products treated at 2.45 GHz the dielectric properties are related to the volumetric water concentration and ionic conductivity (Torringa *et al.*, 2001). Dielectric materials are better absorbers and transmitters of microwaves (Sanga *et al.*, 2000). Thus, if there are not enough polar molecules, in this case water, the food material will have difficulty becoming polarized, at least if polar molecules are added to the product.

Water is a target factor in the dielectric properties of foods; temperature has an effect on the dielectric properties of food, because the loss factor decreases as temperature increases (Kent, 2001). The dielectric constant of food materials decreases with the increase of temperature. Salt or sugar content in the medium is an important factor that has an effect on the dielectric constant and loss factor of the food (Feng, 2000). With respect to the loss factor, at high frequencies, high concentrations generate low levels of dissipated energy.

18.5.3   Milk processing

Microwave energy has been tested in milk to simulate the pasteurization process; batch and continuous systems have been tested to achieve similar inactivation results using conventional thermal treatments (NACMCF, 2006). In

1969, the first research group to study the use of microwave energy for pasteurization of milk (Clare *et al.*, 2005) reported the results of their work, and until today, there have been few reports on the use of this technology for pasteurization of dairy products. This late 1960s research group, led by Hamid, reported that the final quality of milk pasteurized with microwave technology was much better than that of thermal pasteurized milk. Since then, some studies have shown that *Listeria monocytogenes* can be inactivated in milk using microwave energy (71.7ºC for 10 min) (Sarkar, 2006). In an interesting study evaluating different fat contents in milk (2.5, 3, 3.5%), non-fat solids (7.5, 8.5%), and acidity content (1.6, 1.7, 1.8 g/l) in milk samples, *Listeria monocytogenes* biotype 4A took less than 35 s to be inactivated (7 log reductions) using microwave energy at 2450 MHz. The higher the non-fat solids and fat content, the higher the inactivation was as well. Regarding acidity, when lactic acid content was higher the inactivation rate was lower, mainly because the ionic content of the medium increased and the penetration of microwave radiation decreased (Firouzi *et al.*, 2005). Other authors reported inactivation of pathogenic microorganisms (more than 5 log reductions) in milk using microwave energy, as well as inactivation of alkaline phosphatase enzyme (Calvo and Olano, 1992).

Overall quality

Some studies comparing the use of UHT pasteurization and microwave energy to process milk have shown that better sensorial characteristics (aroma, taste, color) are observed after processing (Clare *et al.*, 2005). In the study reported by Valero *et al.* (2000), milk samples were processed with microwave energy (2450 MHz) and two temperatures (80 and 92ºC) for 15 s, while others were conventionally pasteurized with a heat exchanger using the same temperatures and time. Samples processed by microwave at the highest temperature had a longer storage life without off-flavors, and pH remained constant longer than the other samples. Sensorial evaluation also showed higher scores for the microwave-treated samples at 92ºC.

The use of microwave on a smaller scale has been reported in specific cases for warming up breast milk and infant formula; detailed evaluation of vitamins and fatty acids has been tested in microwave products; no changes in immunoglobulin and fatty acids have been observed when the temperature is below 60ºC (Lassen and Ovesen, 1995).

Some volatile compounds in milk (aldehydes, ketones, alcohols, esters, aromatic hydrocarbons) and monosaccharides (galactose, glucose, *myo*-inositol) were quantified in pasteurized milk (using microwave and conventional heat exchanger); concentrations similar to that of the raw milk were found in the treated samples even during the storage period (15 d) at 4.5ºC (Valero *et al.*, 2000).

The mineral balance of milk was tested after heating it with microwave energy. Because of the rotation of bipolar molecules and translation of ionic components generated by microwave energy in the food, the possibility of

reducing the ionic calcium and further coagulation properties in milk after treatment was assessed. However, similar results were found in the mineral content (Ca, Mg, P, and K) of heat- and microwave-treated milks; also, the coagulation time was longer in the microwave-treated sample than in the raw milk sample (De la Fuente *et al.*, 2002).

18.5.4   Regulatory issues

Microwave technology has been used for different purposes in the food industry (e.g. thawing, drying, and cooking), and it has been approved for specific applications. A formal petition to use microwave as a sterilization technology (at 45 kW, 915 MHz; semi-continuous system) was submitted to the FDA on October 2008 and has made important headway (Tang, 2009). Indeed, most of the advantages of using this technology to preserve food have been demonstrated in many of the reported experiments; the next step is dependent upon regulatory officials in their approval of microwave technology as an alternative to pasteurization and sterilization of food, but more studies validating this technology must be completed first.

18.5.5   Advantages and limitations

Using microwave energy to pasteurize milk offers many advantages, for example the characteristic fouling of heat exchangers can be eliminated because of the continuous flow of microwave heating systems. Milk allows for a faster heating process because of the presence of proteins and ions, facilitating the microwave process. Denaturation of proteins is minimized; enzyme inactivation is achieved at the same levels as in conventional thermal treatment, and sensorial characteristics are very similar to those of heat-treated milk (Coronel *et al.*, 2003; De la Fuente *et al.*, 2002). One of the main limitations in microwaving milk is the non-uniformity of heat distribution during the process; indeed, this topic is still under research in many laboratories worldwide.

18.6   Ohmic heating

Ohmic (Joule) heating was introduced in 1840 when its discoverer, James Prescott Joule, generated heat in an electrical conductor (Sastry *et al.*, 2002). In the nineteenth century, ohmic heating was used to heat various materials; in the 1900s, electric pasteurization was used to process milk, by passing the liquid between two plates that exhibited an electrical potential difference (Morrissey and Almonacid, 2005; Castro *et al.*, 2004; Wang and Sastry, 1993). Nevertheless, this technology was abandoned due to the lack of inert materials needed to make the electrodes (Castro *et al.*, 2004). From the 1980s to the present, research and improvement of ohmic heating technology has been ongoing, resulting in a number of new design elements, variables, and materials (Morrissey and

Almonacid, 2005). Ohmic heating is a high temperature short time (HTST) process, and the potential for its use in the food industry is very high; blanching, evaporation, dehydration, fermentation, and pasteurization (Castro *et al.*, 2004) are among its many potential uses.

18.6.1   Definition

The basic principle of ohmic heating is the passage of an alternating electrical current through a food sample (one that has electrical conductivity), wherein the electrical resistance of the food material generates heat (Morrissey and Almonacid, 2005; Icier and Ilicali, 2005; Shirsat *et al.*, 2004; Ruan *et al.*, 2001). The heat instantly generated inside the food is proportional to the square of the current induced (electric field strength), the electrical conductivity (Icier and Ilicali, 2005; Sastry *et al.*, 2002), and the type of food being heated (Ruan *et al.*, 2001). Because the generation of heat and its distribution throughout the material are unusually fast, the food retains its flavor and particulate integrity better than when using conventional thermal treatment (Shirsat *et al.*, 2004). Samples with higher conductivities show higher heating rates; some variations in samples can be observed with differences in specific heat (Ruan *et al.*, 2001). Foods containing both water and salts are capable of conducting electricity (Fellows, 2000); this electrical conductivity of food particles or liquids increases linearly with temperature (Imai *et al.*, 1995). Moreover, electrical conductivity is influenced by ionic content, so by adjusting the level of ions (salts) in the product, over-processing can be avoided. It is also important to know that fats and syrups are electrical insulators, while brines, pickles, and acidic solutions have high conductivities (Ruan *et al.*, 2001). To increase effectiveness, electrical conductivity can be enhanced through electro-osmosis (an electric field, by using an alternating current field or applying an alternating field over the liquid membrane) (Halden *et al.*, 1990). In comparison, ohmic heating is a less aggressive thermal treatment (Castro *et al.*, 2004). Even though electrical conductivity increases with temperature and ohmic heating is more effective at higher temperatures, in some products heating is more uniform than in microwave technology (Sastry *et al.*, 2002). Ohmic heating is also known as 'resistance heating' or 'direct resistance heating' (Ruan *et al.*, 2001), as well as 'electroheating' (Fellows, 2000). Above all, ohmic heating is a viable alternative method for use in cooking and sterilizing pumpable foods; for example, it can be used as a continuous line heater for viscous liquids and food mixtures (Icier and Ilicali, 2005).

18.6.2   Mode of action

Ohmic heating has been used in the food industry with different goals in mind. One of its main uses is related to sterilization processes, but problems still exist that need to be addressed. In particular, problems can occur in samples with solid and liquid phases since electrical conductivity is different in each phase

(Sastry *et al.*, 2002). Nevertheless, this technology is quite capable of generating enough energy to sterilize or pasteurize food products effectively (Ruan *et al.*, 2001).

Complete information about microbial inactivation is not available, but some studies have been carried out; however, these cases are not detailed. A comparison of thermal treatment and ohmic heating to inactivate *Zygosaccharomyces bailii* showed no differences (Sastry *et al.*, 2002). In the case of *Escherichia coli*, a mild electrical pretreatment decreased the inactivation requirements (Sastry *et al.*, 2002).

The mode of action of ohmic heating in the inactivation of cells is related to electroporation, with the occurrence of pore-forming mechanisms during treatment. The low frequency of ohmic heating (50–60 Hz) is responsible for the inactivation of microorganisms because it allows cell walls to build up charges and form pores. For example, a two-stage ohmic heating treatment (ohmic heating, followed by heat treatment) was effective in the inactivation of *Bacillus subtilis* spore cells. In studies on *Saccharomyces cerevisiae* inactivation, leakage of internal constituents was observed when cells were submitted to ohmic heating (Sastry *et al.*, 2002).

Nevertheless, some studies on electropasteurization of milk conducted at the beginning of the last century present different data. In this process, the application of electrical current through milk generated heat; this application was reported as being responsible for bacterial death. Other research has concluded that the only effect during treatment is the generated heat from the electrical current, without any additional effect from electricity. In experiments with high voltage (i.e., pulsed electric fields) bacterial death is due to loss of cellular membrane function as a semipermeable barrier. Permanent membrane damage is related to high voltage discharges, whereas with low voltage the killing effect is not fully understood. Some studies on yeasts suggest that the use of low voltage alternating current could generate the formation of toxic substances, such as free chlorine or hydrogen peroxide, which could be the cause of cell death. The flow of low voltage alternating current in a medium such as a food can generate enough heat to inactive bacteria cells (Palaniappan *et al.*, 1990).

18.6.3   Milk processing

As a novel technology, there are very few reports regarding use of ohmic heating to pasteurize milk. Some of these reports are related to inactivation of mesophilic bacteria and protein denaturation (Sun *et al.*, 2008), while other studies mention the changes in fatty acid content and changes in the fat globule membrane after processing (Pereira *et al.*, 2008). However, in a study related to pasteurization, the authors asserted that processing time is shorter in ohmic heating pasteurization compared with conventional pasteurization; for example, mesophilic bacteria and *Streptococcus thermophilus* were inactivated faster in skim milk under ohmic heating compared with thermal treatment at the same

temperature and processing times. Furthermore, no changes in protein content were detected after processing (Sun *et al.*, 2008). Indeed, these results are important, but from a microbiological point of view, experiments testing the inactivation of pathogenic bacteria such as *Escherichia coli*, *Salmonella*, and *Listeria monocytognes* are highly desirable using ohmic heating. In fact, although ohmic heating technology has shown slow development for use in milk pasteurization, it is beginning to be explored again. Today, with new materials available for electrodes and better process control systems, positive results with this technology could be demonstrated in the near future.

18.6.4   Regulatory issues
Today, ohmic heating is used quite differently in the food industry apart from pasteurization or sterilization. Some of the current uses include baking, drying, cooking, thawing, diffusion, and extraction. However, more information and better materials for equipment could enhance this technology and speed the way for its approval as a potential pasteurization or sterilization method.

18.6.5   Advantages and limitations
The alleged advantages of new thermal technologies like ohmic heating and microwave energy as seen in the resulting low loss of nutrients are based on kinetics parameters (k, z, E_a). In the case of ohmic heating, temperature increases rapidly in only a few seconds, with simultaneous inactivation of bacteria, but more time would be needed to promote chemical reactions such that nutrients are affected. One example is a lethality of $F_0 = 8$ in conventional heating at 130°C, producing a cook value of $C_0 = 8$ (thiamin degradation); with ohmic heating the F_0 value is 24 at 140°C and $C_0 = 4$ (Ruan *et al.*, 2001). Also, from the point of view of activation energy, sterilization reactions have higher values than those related to product degradation (Schreier *et al.*, 1993).

   Some advantages of ohmic heating follow:

- Heating food materials volumetrically by internal heat generation
- Particulate temperatures similar to or higher than liquid temperatures
- Minimal mechanical damage
- Better nutrient and vitamin retention
- Lower fouling in heat transfer surface
- High energy efficiency (90%)
- Optimization of capital investment
- Ease of process control
- Faster heating of food (1°C/s)
- Heat transfer coefficients do not limit rate of heating
- High temperatures reached (UHT)
- Suitable for continuous processing
- Uniform heating of particulate foods

(Ruan *et al.*, 2001; Fellows, 2000; Wang and Sastry, 1993).

18.7   Ultraviolet light

Radiation is the propagation of energy through space. There is more than one kind of radiative energy, each having a different wavelength in the electromagnetic spectrum with specific applications, such as microwaves, radio waves, and gamma radiation. Another example is high intensity light, a disinfection method that uses intense white light (Butz and Tauscher, 2002). The wavelengths of the electromagnetic spectrum used in high intensity light come from the ultraviolet to near infrared region (Barbosa-Cánovas *et al.*, 1998). The food industry has used pulses of light (flashes applied in seconds) to inactivate microorganisms (Butz and Tauscher, 2002) in the past, but today the most common application of this high intensity energy is ultraviolet light applied without pulses, with a minimum of visible light (Ohlsson, 2002). However, in recent reports, pulsed UV light treatment appears to be more effective in microbial inactivation (Krishnamurthy *et al.*, 2008b). Furthermore, ultraviolet light can be applied to inactivate harmful microorganisms at low temperatures (Tran and Farid, 2004; López-Malo and Palou, 2005).

18.7.1   Definition

Ultraviolet radiation is in the range 100 to 400 nm in the electromagnetic spectrum (Fig. 18.1), and each range of UV has different applications. Based on the wavelength, there are four types of ultraviolet radiation, UV-A, UV-B, UV-C, and UV-V, as described by Guerrero-Beltrán and Barbosa-Cánovas (2004). UV-A (long wavelength) ranges from 320 to 400 nm and can tan human skin. UV-B (medium wavelength) ranges from 280 to 320 nm and can burn the skin. UV-C (short wavelength) ranges from 200 to 280 nm and has a germicidal effect. Finally, UV-V (shortest wavelength) ranges from 100 to 200 nm and corresponds to the vacuum UV range.

In the above classification of ultraviolet light, UV-C (200–280 nm) is the radiation of most interest in food processing because of its germicidal effect, although some authors believe the higher 250–280 nm range is more applicable to inactivating bacteria (Tran and Farid, 2004). Energy doses used are at least 400 J/m^2, and critical factors include the transmissivity of the product, geometry, power, and product flow profile, among others (Butz and Tauscher, 2002). Reducing the microbial load on surfaces by means of ultraviolet light has great potential (Wong *et al.*, 1998).

18.7.2   Mode of action

Ultraviolet equipment is very simple. The main component is the source of radiation, the ultraviolet lamp. The most commonly used is the low-pressure mercury vapor germicidal lamp. UV radiation is measured with radiometers, and the quantity of energy is expressed as W/m^2. Other parts of the equipment include the concentric tubes around the lamp, serving as a liquid container, the refrigeration systems, and pumps (Guerrero-Beltrán and Barbosa-Cánovas,

2004). The sample flows through the concentric tubes around the radiation source, with flow rate dependent on the dose applied to a specific product; sometimes a quartz tube covers the lamp, allowing the transmission of energy. However, since quartz is very expensive, other types of glass are used for the same purpose (López-Malo and Palou, 2005). Researchers at Rutgers University validated quartz tubes for use in achieving and guaranteeing 5 log reductions in the counts of specific microorganisms such as *Escherichia coli* (Vasavada, 2003) according to FDA requirements for pasteurization processes. Pulsed UV light has been shown to be more effective in microbial inactivation than continuous UV light (NACMCF, 2006).

   Although the wavelength considered most lethal against bacteria, viruses, protozoa, molds, yeasts and algae is between 220 and 300 nm, the maximum effect is observed between 250 and 270 nm; as a result, ultraviolet lamps use 254 nm as a standard wavelength (Guerrero-Beltrán and Barbosa-Cánovas, 2004). Ultraviolet treatment in water allows a 99.999% microbial reduction after 60 s of treatment (López-Malo and Palou, 2005). This is in accordance with the 5 log reductions required by the FDA for approval of a pasteurization process.

   Variables that influence the microbial effect of UV-C include type of microorganism, media, initial count, and stage of the microorganism. In studies on the inactivation of microorganisms with UV-C, the most resistant appears to be molds and yeasts (Guerrero-Beltrán and Barbosa-Cánovas, 2004). This is because of the pigments present in some microorganisms. For example, in molds such as *Aspergillus niger*, which contain dark pigments, resistance to UV light is higher than in molds without dark pigments (Marquenie *et al.*, 2003). The mechanism of inactivation in microorganisms is a result of direct damage to the DNA (deoxyribonucleic acid). Its transcription and replication are blocked, resulting in the death of cells. Moreover, mutations can occur in some injured cells via dimerization of the pyrimidine bases in DNA. Pyrimidine and purine bases have the ability to absorb energy in this wavelength (Marquenie *et al.*, 2003; López-Malo and Palou, 2005). This is because ultraviolet light contains high energy photons (Wong *et al.*, 1998). Some cells can repair the damage in their structures through some proteins and reactivate them when the light wavelength changes (Guerrero-Beltrán and Barbosa-Cánovas, 2004). Other effects associated with UV inactivation are related to changes in the permeability of the cell membrane, with the consequent loss of electrolytes, amino acids and carbohydrates (Marquenie *et al.*, 2003). Resistance to UV light can increase depending on the type of microorganism, the most resistant being Gram-negative and Gram-positive microorganisms, yeasts, bacterial spores, molds and viruses (López-Malo and Palou, 2005). The required dose depends on many factors, and therefore the same bacteria (e.g., *E. coli*) show different values.

18.7.3   Milk processing

Even though UV light is more effective applied to clear and transparent fluids, some reports demonstrating microbial inactivation in milk can be found in the

literature. For example, monochromatic pulsed UV light was found useful in the inactivation of some bacteria in milk (NACMCF, 2006). *Escherichia coli* ATCC 25922 was inactivated in skim milk, but under different processing conditions that involved higher flow rates and changes in the geometry of the equipment (Milly *et al.*, 2007). When the same culture was used in whole milk, and a dose of 5.8 mJ/cm^2 applied, only 0.73 log reductions of *Escherichia coli* were achieved. However, using an 'accumulated' dose of UV radiation (around 15.8 mJ/cm^2) in goat milk, the inactivation of *Listeria monocytogenes* was increased to achieve a 5 log reduction (Matak *et al.*, 2005). On the other hand, using these accumulated doses to inactivate bacteria can generate undesirable changes in milk, such as odor and oxidative and hydrolytic rancidity (Matak *et al.*, 2007). Since the opacity of milk limits the penetration of UVC, most of the microorganisms cannot receive the same dose (Milly *et al.*, 2007). The absorption coefficient (cm^{-1}) for raw milk is 290 at 253.7 nm, for water 0.01; in other words, the penetration for 90% water absorption is 100 cm, for raw milk only 0.003 cm (Koutchma, 2009). Also, the presence of proteins and fat globules in milk can behave as a shadow, protecting the microorganisms from UV radiation (Milly *et al.*, 2007). However, if a turbulent fluid system is used that has high speed flows, allowing longer exposure time to UV radiation, and a mix of all fluid is achieved, then UV light is an option to pasteurize milk.

Mycobacterium avium subsp. *paratuberculosis* was studied under UV treatment in buffer and skim (1.7%) and whole milk (4%), showing great resistance to inactivation, especially in the two milks. Using extremely high doses of UV radiation (1000 mJ/ml) only a 0.5 to 1 log reduction could be achieved, generating undesirable effects in the sensorial quality of the milks (Altic *et al.*, 2007). *Staphylococcus aureus* was inactivated in raw milk treated under UV treatment, using light pulses and at various distances from the quartz window in the UV lamp. Up to 8 log reductions in the sample were achieved using 180 s of radiation at a distance of 8 cm from the quartz window. Because of processing conditions in some experiments, there was an increase in temperature (90ºC) that could have an effect on microbial inactivation (Krishnamurthy *et al.*, 2008b). In another study mesophilic bacteria were inactivated (3 log reduction) in milk using 15 kJ/l, as well as coliforms in a high ratio, as opposed to spore-forming microorganisms that did not show high inactivation under UV treatment (Altic *et al.*, 2007).

Smith *et al.* (2002) reported an interesting study using a mix of different microorganisms inoculated in bovine milk and treated under pulsed UV laser light. Cells of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella choleraesuis* serotype Dublin, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Aeromonas hydrophilia*, and *Serratia marcescens* were inoculated in raw milk; samples exposed at 25 J/cm^2 of pulsed UV intensity did not show growth of any bacteria.

As a novel technology there are very few reports regarding use of UV light in processing milk; most are focused on microbial inactivation. Information regarding physicochemical changes in nutritive or sensorial quality is very

limited. Because of the action principle of UV treatment some photodegradation reactions could be the case with some organic molecules in the product, but enough energy must be applied to break or bind these molecules (Koutchma, 2009). Vitamin A, carotene, and riboflavin are nutrients commonly present in milk; because of their photosensitivity, they could be affected by UV radiation if treatment involves extremely high energy.

18.7.4   Advantages and limitations

This technology has a number of advantages worth mentioning. For one, it is a 'clean' technology that does not generate chemical residues. It is an economic option for disinfection compared with common processing methods. UV light is a cold and dry process, and since inactivation of microorganisms is a result of radiation energy, there are no undesirable changes in the food's quality properties (Guerrero-Beltrán and Barbosa-Cánovas, 2004; Wong *et al.*, 1998). Furthermore, the combination of treatment times and low or high intensity UV light can achieve high microbial reduction.

   Despite these advantages, application of this technology is limited to surfaces or clear materials such as water, air, and polyethylene. Radiation does not penetrate the product deeply, but nevertheless some modifications could be made to the system. Ultraviolet light is not very effective in porous and rugged surface foods because they generate shadows. These shadows act as shield to the UV light. Furthermore, bacteria show higher resistance in liquids than in the air.

18.8   Other available technologies

Although more time has been devoted to researching the above-mentioned technologies as to their potential in the dairy industry, there are a number of available technologies that may be useful for other food products. Some are undergoing more basic research, and just starting the setup and basis of a new technology.

   One of these novel technologies is radio frequency, which is currently used in the food industry for specific operations but is still under research for microbial inactivation. Radio frequency is another technique of dielectric heating that is quite similar to the principles of microwave technology. Heat is generated inside the product, resulting from the polarization of molecules and migration of ions that occurs at high frequency (Wang *et al.*, 2003). The advantage of radio frequency over microwave energy is that penetration depth is deeper due to the frequency. The selected frequencies for industrial, domestic, scientific, and medical applications are 13.56, 27.12, and 40.68 MHz (Awuah *et al.*, 2005). The study of microbial inactivation in milk using radio frequency had important results. *Listeria innocua* and *Escherichia coli* K-12 were inactivated by 5 and 7 log reductions, respectively, using a 2 kW, 27.12 MHz device; total residence time was 55 s (Awuah *et al.*, 2005), proving how fast the treatment could be.

Other interesting studies have been conducted in milk using infrared radiation, which is part of the electromagnetic spectrum (Fig. 18.1), with applications ranging from 0.5 to 1000 μm (Krishnamurthy *et al.*, 2008a). Although infrared is currently used for other applications, some studies related to microbial inactivation in food have shown the effects of this radiation on DNA, RNA, and other cellular components of bacteria. Some of the bacteria studied under infrared treatment are *Escherichia coli* O157:H7 (Sawai *et al.*, 1995), lactic acid bacteria, coliforms (Giraffa and Bossi, 1984), and more recently *Staphylococcus aureus* (Krishnamurthy *et al.*, 2008a) which showed total inactivation in milk after 4 min at 619ºC (lamp temperature).

18.9   Conclusions

Several milk processing alternatives have been discussed in this chapter, some of which have had a higher degree of research with alleged advantages that could benefit the dairy industry. Some novel technologies are still establishing the fundamentals of their use in the dairy industry but could be effective as pasteurization alternatives in the coming years. It is clear that most of these novel technologies offer important reductions in processing times and supplied energy, with the added advantage of a milk product that retains its freshness longer.

18.10   References

ALTIC LC, ROWE MT and GRANT IR (2007), 'UV light inactivation of *Mycobacterium avium* subs. *paratuberculosis* in milk as assessed by FASTPlaqueTB phage assay and culture', *Appl Environ Microb*, 73(11): 3728–3733.

ANANTA E, VOIGT D, ZENKER M, HEINZ V and KNORR D (2005), 'Cellular injuries upon exposure of *Escherichia coli* and *Lactobacillus rhamnosus* to high-intensity ultrasound', *J Appl Microbiol*, 99: 271–278.

ASHRAF-CHAUDRY M, BIBI N, KHAN M, KHAN M, BADSHAH A and QHRESHI MJ (2004), 'Irradiation treatment of minimally processed carrots for ensuring microbiological safety', *Radiat Phys Chem*, 71: 169–173.

AWUAH GB, RAMASWAMY HS, ECONOMIDES A and MALLIKARJUNAN K (2005), 'Inactivation of *Escherichia coli* K-12 and *Listeria innocua* in milk using radio frequency (RF) heating', *Innov Food Sci Emerg*, 6: 296–402.

BARBOSA-CÁNOVAS GV, POTHAKAMURY UR, PALOU E and SWANSON BG (1998), *Nonthermal Preservation of Foods*, New York, Marcel Dekker.

BERMÚDEZ-AGUIRRE D and BARBOSA-CÁNOVAS GV (2008a), 'Study of butter fat content in milk on the inactivation of *Listeria innocua* ATCC 51742 by thermo-sonication', *Innov Food Sci Emerg*, 9(2): 176–185.

BERMÚDEZ-AGUIRRE D and BARBOSA-CÁNOVAS GV (2008b), 'Scanning electron microscopy of thermo-sonicated *Listeria innocua* cells', in Gutiérrez-López G, Barbosa-Cánovas GV, Welti-Chanes J and Parada-Arias E, *Food Engineering: Integrated*

Approaches, New York, Springer, 287–294.

BERMÚDEZ-AGUIRRE D, MAWSON R and BARBOSA-CÁNOVAS GV (2008), Microstructure of fat globules in whole milk after thermo-sonication treatments. *J Food Sci*, 73(7): E325–E332.

BERMÚDEZ-AGUIRRE D, MAWSON R, VERSTEEG K and BARBOSA-CÁNOVAS GV (2009), 'Composition parameters, physical–chemical characteristics and shelf-life of whole milk after thermal and thermo-sonication treatments', *J Food Quality*, 32: 283–302.

BUTZ P and TAUSCHER B (2002), 'Emerging technologies: chemical aspects', *Food Res Int*, 35: 279–284.

CABEZA MC, ORDÓÑEZ JA, CAMBERO I, DE LA HOZ L and GARCÍA ML (2004), 'Effect of thermoultrasonication on *Salmonella enterica* serovar *Enteriditis* in distilled water and intact shell eggs', *J Food Protect*, 67(9): 1886–1891.

CALVO MM and OLANO A (1992), 'Review: Thermal treatments of goat's milk', *Rev Esp de Cien Tec Ali*, 32(2): 139–152.

CAMERON M, MCMASTER LD and BRITZ TJ (2008), 'Electron microscopic analysis of dairy microbes inactivated by ultrasound', *Ultrason Sonochem*, 15: 960–964.

CAMERON M, MCMASTER LD and BRITZ TJ (2009), 'Impact of ultrasound on dairy spoilage microbes and milk components', *Dairy Sci Technol*, 89: 83–98.

CARCEL JA, BENEDITO J, SANJUAN N and SÁNCHEZ E (1998), 'Aplicación de los ultrasonidos en las industrias de productos lácteos y derivados', *Alimentación, Equipos y Tecnología*, 135–141.

CASTRO I, TEIXEIRA JA, SALENGKE S, SASTRY SK and VICENTE AA (2004), 'Ohmic heating of strawberry products: electrical conductivity measurements and ascorbic acid degradation kinetics', *Innov Food Sci Emerg*, 5: 27–36.

CHAWLA SP, CHANDER R and SHARMA A (2009), 'Antioxidant properties of Maillard reaction products obtained by gamma-irradiation of whey proteins', *Food Chem*, 116: 122–128.

CLARE DA, BANG WS, CARTWRIGHT G, DRAKE MA, CORONEL P and SIMUNOVIC J (2005), 'Comparison of sensory, microbiological and biochemical parameters of microwave versus indirect UHT fluid skim milk during storage', *J Dairy Sci*, 88: 4172–4182.

CORONEL P, SIMUNOVIC J and SANDEEP KP (2003), 'Temperature profiles within milk after heating in a continuous-flow tubular microwave system operating at 915 MHz', *J Food Sci*, 68(6): 1976–1981.

CRAWFORD LM and RUFF EH (1996), 'A review of the safety of cold pasteurization through irradiation', *Food Control*, 7(2): 87–97.

D'AMICO DJ, SILK TM, WU J and GUO M (2006), 'Inactivation of microorganisms in milk and apple cider treated with ultrasound', *J Food Protect*, 69(3): 556–563.

DE LA FUENTE MA, OLANO A and JUÁREZ M (2002), 'Mineral balance in milk heated using microwave energy', *J Agric Food Chem*, 50: 2274–2277.

DROUZAS AE, TSAMI E and SARAVACOS GD (1999), 'Microwave/vacuum drying of model fruit gels', *J Food Eng*, 39: 117–122.

EARNSHAW RG, APPLEYARD J and HURST RM (1995), 'Understanding physical inactivation processes: Combined preservation opportunities using heat, ultrasound and pressure', *Int J Appl Microbiol*, 28: 197–219.

EHLERMANN DAE (2002a), 'Irradiation', in Henry CJK and Chapman C, *The Nutrition Handbook for Food Processors*, Boca Raton, FL, CRC Press.

EHLERMANN DAE (2002b), 'Microwave Processing', in Henry CJK and Chapman C, *The Nutrition Handbook for Food Processors*, Boca Raton, FL, CRC Press.

FELLOWS P (2000), *Food Processing Technology. Principles and Practice*, 2nd edn, Boca Raton, FL, CRC Press.

FENG H (2000), 'Microwave drying of particulate foods in a spouted bed', PhD Dissertation, Washington State University, Pullman, WA.

FIROUZI R, SHEKARFOROUSH SS and MOJAVER S (2005), 'Destruction effect of microwave radiation on *Listeria monocytogenes* biotype 4A in milk', *Adv Food Sci*, 27(3): 131–134.

FURUTA M, YAMAGUCHI M, TSUKAMOTO T, YIM B, STAVARACHE CE, HASIBA K and MAEDA Y (2004), 'Inactivation of *Escherichia coli* by ultrasonic irradiation', *Ultrasonics Sonochem*, 11(2): 57–60.

GIRAFFA G and BOSSI MG (1984), Trattamento del latte con UV ed IR+UV: Effetto su microflora e attitudine alla coagulazione. *Latte*, 9(6): 476–488.

GUERRERO S, TOGNON M and ALZAMORA SM (2005), 'Response of *Saccharomyces cerevisiae* to the combined action of ultrasound and low weight chitosan', *Food Control*, 16: 131–139.

GUERRERO-BELTRÁN JA and BARBOSA-CÁNOVAS GV (2004), 'Review: Advantages and limitations on processing foods by UV light', *Food Sci Technol Int*, 10(3): 137–147.

HALDEN K, DE ALWIS AAP and FRYER PJ (1990), 'Changes in the electrical conductivity of foods during ohmic heating', *Int J Food Sci Tech*, 25: 9–25.

HAYTA M, ALPASLAN M and BAYSAR A (2002), 'Effect of drying methods on functional properties of Tarhana: a wheat flour–yogurt mixture', *J Food Sci*, 67(2): 740–744.

ICIER F and ILICALI C (2005), 'The use of tylose as a food analog in ohmic heating studies', *J Food Eng*, 69(1): 67–77.

IMAI T, UEMURA K, ISHIDA N, YOSHIZAKI S and AKINORI N (1995), 'Ohmic heating of Japanese white radish *Rhaphanus sativus* L.', *Int J Food Sci Tech*, 30: 461–472.

JANDAL JM (1990), 'Use of radiation in milk and milk products', *Agr Rev,* 11(2), 109–116.

KADDOURI H, MIMOUN S, EL-MECHERFI KE, CHEKROUN A, KHEROUA O and SAIDI D (2008), 'Impact of *gamma*-radiation on antigenic properties of cow's milk *beta*-lactoglobulin', *J Food Protect*, 71(6): 1270–1272.

KENT M (2001), 'Microwave measurements of product variables', in Kress-Rogers E and Brimelow CJB, *Instrumentation and Sensors for the Food Industry*, 2nd edn, Boca Raton, FL, CRC Press.

KING CJ (1973), 'Freeze-drying', in Van Arsdel WB, Copley MJ and Morgan AI, *Food Dehydration*, 2nd edn, Westport, CT, Avi Publishing, Vol. I, 191–192.

KOMAROV V, WANG S and TANG J (2004), 'Permittivity and measurement', in Chang K, *The Wiley Encyclopedia of RF and Microwave Engineering*, New York, John Wiley & Sons, Vol. 4, 3693–3711.

KOUTCHMA T (2009), 'Advances in ultraviolet light technology for non-thermal processing of liquid foods', *Food Bioprocess Technol*, 2: 138–155.

KRISHNAMURTHY K, JUN S, IRUDAYARAJ J and DEMIRCI A (2008a), 'Efficacy of infrared heat treatment for inactivation of *Staphylococcus aureus* in milk', *J Food Process Eng*, 31: 798–816.

KRISHNAMURTHY K, DEMIRCI A and IRUDAYARAJ J (2008b), 'Inactivation of *Staphylococcus aureus* in milk and milk foam by pulsed UV-light treatment and surface response modeling', *T ASABE*, 51(6): 2083–2090.

LASSEN A and OVESEN L (1995), 'Nutritional effects of microwave cooking', *Nutrition Food Sci*, 4: 8–10.

LÓPEZ-MALO A and PALOU E (2005), 'Ultraviolet light and food preservation', in Barbosa-

Cánovas GV, Tapia MS and Cano MP, *Novel Food Processing Technologies,* Boca Raton, FL, CRC Press, 405–421.

MAÑAS P, PAGÁN R and RASO J (2000), 'Predicting lethal effect of ultrasonic waves under pressure treatments on *Listeria monocytogenes* ATCC 15313 by power measurements', *J Food Sci*, 65(4): 663–667.

MARQUENIE D, GERAERD AH, LAMMERTYN J, SOONTJENS C, VAN IMPE JF, MICHIELS CW and NICOLAÏ BM (2003), 'Combinations of pulsed white light and UV-C or mild heat treatment to inactivate conidia of *Botrytis cinerea* and *Monilia fructigena*', *Int J Food Microbiol*, 85: 185–196.

MASKAN M (2000), 'Microwave/air and microwave finish drying of banana', *J Food Eng*, 44: 71–78.

MATAK KE, CHUREY JJ, WOROBO RW, SUMNER SS, HOVINGH E, HACKNEY CR and PIERSON MD (2005), 'Efficacy of UV light for the reduction of *Listeria monocytogenes* in goat's milk', *J Food Protect*, 68(10): 2212–2216.

MATAK KE, SUMNER SS, DUNCAN SE, HOVINGH E, WOROBO RW and HACKNEY CR (2007), 'Effects of ultraviolet irradiation on chemical and sensory properties of goat milk', *J Dairy Sci*, 90: 3178–3186.

MCCLEMENTS DJ (1995), 'Advances in the application of ultrasound in food analysis and processing', *Trends Food Sci Tech*, 6(9): 293–299.

MILLY PJ, TOLEDO RT, CHEN J and KAZEM B (2007) 'Hydrodynamic cavitation to improve bulk fluid to surface mass transfer in a nonimmersed ultraviolet system for minimal processing of opaque and transparent fluid foods', *J Food Sci*, 72(9): M407–M413.

MOLINS RA (2001), 'Introduction', in Molins RA, *Food Irradiation: Principles and Applications*, New York, John Wiley & Sons, 1–21.

MOREHOUSE KM and KOMOLPRASERT V (2004), 'Irradiation of food and packaging: an overview', in Komolprasert V and Morehouse KM, *Irradiation of Food and Packaging*, Washington, DC, American Chemical Society, 1–11.

MORRISSEY MT and ALMONACID S (2005), 'Rethinking technology transfer', *J Food Eng*, 67(1–2): 135–145.

MOY JH (2005), 'Food irradiation – an engineering technology', in Barbosa-Cánovas GV, Tapia MS and Cano MP, *Novel Food Processing Technologies*, Boca Raton, FL, CRC Press, 375–404.

NACMCF (2006), 'Requisite scientific parameters for establishing the equivalence of alternative methods of pasteurization', National Advisory Committee on Microbiological Criteria for Foods, *J Food Protect*, 69(5): 1190–1216.

NAGHMOUSH MR, SHEHATA TE, GIRGIS ES, KAMAL TH and IBRAHIM MK (1983), 'Gamma irradiation effect on some bacteriological and other properties of cow's, buffaloes and goat's milk', *Egyptian J Dairy Sci*, 11: 61–69.

NOCI F, WALKING-RIBEIRO M, CRONIN DA, MORGAN DJ and LYNG JG (2009), 'Effect of thermo-sonication, pulsed electric field and their combination on inactivation of *Listeria innocua* in milk', *Int Dairy J*, 19: 30–35.

OHLSSON T (2002), 'Minimal processing of foods with non-thermal methods', in Ohlsson T and Bengtsson N, *Minimal Processing Technologies in the Food Industry*, Boca Raton, FL, CRC Press.

OKOS MR, NARSIMHAN G, SINGH RK and WEITNAUER AC (1992), 'Food dehydration', in Heldman DR and Lund DB, *Handbook of Food Engineering*, New York, Marcel Dekker, 437–562.

OSAILI TM, SHAKER RR, AL-HASAN ASA, AYYASH MM and MARTIN EM (2007), 'Inactivation of

Enterobacter sakazakii in infant milk formula by gamma irradiation: Determination of D_{10}-value', *J Food Sci*, 72(3): M85–M88.

OSAILI TM, AL-NABULSI AA, SHAKER RR, AYYASH MM, OLAIMAT AN, AL-HASAN ASA, KADORA KM and HOLLEY RA (2008), 'Effects of extended dry storage of powdered infant milk formula on susceptibility of *Enterobacter sakazakii* to hot water and ionizing radiation', *J Food Protect*, 71(5): 934–939.

PAGÁN R, MAÑAS P, ALVAREZ I and CONDÓN S (1999), 'Resistance of *Listeria monocytogenes* to ultrasonic waves under pressure at sublethal (manosonication) and lethal (manothermosonication) temperatures', *Food Microbiol*, 16: 139–148.

PALANIAPPAN S, SASTRY SK and RICHTER ER (1990), 'Effects of electricity on microorganism: A review', *J Food Process Pres*, 14: 393–414.

PATIL BS (2004), 'Irradiation applications to improve functional and components of fruits and vegetables', in Komolprasert V and Morehouse KM, *Irradiation of Food and Packaging*, Washington, DC, American Chemical Society, 117–137.

PELCZAR MJ and REID RD (1972), *Microbiology*, New York, McGraw-Hill, 783–807.

PEREIRA RN, MARTINS RC and VICENTE AA (2008), 'Goat milk free fatty acid characterization during conventional and ohmic heating pasteurization', *J Dairy Sci*, 91: 2925–2937.

PIOTROWSKI D, LENART A and WARDZYŃSKI A (2004), 'Influence of osmotic dehydration on microwave-convective drying of frozen strawberries', *J Food Eng*, 65: 519–525.

RAMASWAMY HS, KOUTCHMA T and TAJCHAKAVIT S (2001), 'Enhanced thermal effects under microwave heating conditions', in Welti-Chanes J, Barbosa-Cánovas GV and Aguilera JM, *Engineering and Food for the 21st Century*, Boca Raton, FL, CRC Press.

REGIER M and SCHUBERT H (2001), 'Microwave processing', in Richardson P, *Thermal Technologies in Food Processing*, Boca Raton, FL, CRC Press.

RUAN R, YE X and CHEN P (2001), 'Ohmic heating', in Richardson P, *Thermal Technologies in Food Processing*, Boca Raton, FL, CRC Press.

SANGA E, MUJUMDAR AS and RAGHAVAN GSV (2000), 'Principles and applications of microwave drying', in Mujumdar AS, *Drying Technology in Agriculture and Food Science*, Enfield, NH, Science Publishers, 253–289.

SARKAR S (2006), 'Shelf-life extension of cultured milk products', *Nutrition Food Sci*, 36(1): 24–31.

SASTRY SK, YOUSEF A, CHO H-Y, UNAL R, SALENGKE S, WANG W-C, LIMA M, KULSHRESTHA S, WONGSA-NGASRI P and SENSOY I (2002), 'Ohmic heating and moderate electric field (MEF) processing', in Welti-Chanes J, Barbosa-Cánovas GV and Aguilera JM, *Engineering and Food for the 21st Century*, Boca Raton FL, CRC Press.

SATIN M (1996), *Food Irradiation*, Lancaster, PA, Technomic Publishing Co.

SAWAI J, SAGARA K, IGARASHI H, HASHIMOTO A, KOKUGAN T and SHIMIZU M (1995), 'Injury of *Escherichia coli* in physiological phosphate buffered saline induced by far-infrared irradiation', *J Chem Eng Jpn*, 28(3): 294–299.

SCHREIER PJR, REID DG and FRYER PJ (1993), 'Enhanced diffusion during the electrical heating of foods', *Int J Food Sci Tech*, 28: 249–260.

SHIRSAT N, LYNG JG, BRUNTON NP and MCKENNA B (2004), 'Ohmic processing: Electrical conductivities of pork cuts', *Meat Sci*, 67: 507–514.

SMITH WL, LAGUNAS-SOLAR MC and CULLOR JS (2002), 'Use of pulsed ultraviolet laser light for the cold pasteurization of bovine milk', *J Food Protect*, 65(9): 1480–1482.

SUN H, KAWAMURA S, HIMOTO J, ITOH K, WADA T and KIMURA T (2008), 'Effects of ohmic heating on microbial couints and denaturation of proteins in milk', *Food Sci Tech*

Res, 14(2): 117–123.

TANG J (2009), personal communication, Washington State University, Pullman, WA.

TORRINGA E, ESVELD E, SCHEEWE I, VAN DEN BERG R and BARTELS P (2001), 'Osmotic dehydration as a pre-treatment before combined microwave–hot-air drying of mushrooms', *J Food Eng*, 49: 185–191.

TRAN MTT and FARID M (2004), 'Ultraviolet treatment of orange juice', *Innov Food Sci Emerg*, 5: 495–502.

TSUKAMOTO I, CONSTANTINOIU E, FURUTA M, NISHIMURA R and MAEDA Y (2004a), 'Inactivation effect of sonication and chlorination on *Saccharomyces cerevisiae*. Calorimetric analysis', *Ultrasonics Sonochem*, 11: 167–172.

TSUKAMOTO I, YIM B, STAVARACHE CE, FURUTA M, HASHIBA K and MAEDA Y (2004b), 'Inactivation of *Saccharomyces cerevisiae* by ultrasonic irradiation', *Ultrasonics Sonochem*, 11: 61–65.

UGARTE-ROMERO E, FENG H, MARTIN SE, CADWALLADER KR and ROBINSON SJ (2006), 'Inactivation of *Escherichia coli* with power ultrasound in apple cider, *J Food Sci*, 71(2): E102–E108.

UGARTE-ROMERO E, FENG H and MARTIN SE (2007), 'Inactivation of *Shigella boydii* 18 IDPH and *Listeria monocytogenes* Scott A with power ultrasound at different acoustic energy densities and temperatures', *J Food Sci*, 72(4): M103–M107.

URBAIN WM (1986), *Food Irradiation*, Orlando, FL, Academic Press.

VALERO E, VILLAMIEL M, SANZ J and MARTÍNEZ-CASTRO I (2000), 'Chemical and sensorial changes in milk pasteurized by microwave and conventional systems during cold storage', *Food Chem*, 70: 77–81.

VASAVADA PC (2003), 'Alternative processing technologies for the control of spoilage bacteria in fruit juices and beverages', in Foster T and Vasavada PC, *Beverage Quality and Safety*, Boca Raton, FL, CRC Press.

VILLAMIEL M and DE JONG P (2000), 'Influence of high-intensity ultrasound and heat treatment in continuous flow on fat, proteins, and native enzymes of milk', *J Agr Food Chem*, 48: 472–478.

VILLAMIEL M, VAN HAMERSVELD EH and DE JONG P (1999), 'Review: Effect of ultrasound processing on the quality of dairy products', *Milchwissenschaft*, 54(2): 69–73.

WANG WC and SASTRY SK (1993), 'Salt diffusion into vegetable tissue as a pretreatment for ohmic heating: electrical conductivity profiles and vacuum infusion studies', *J Food Eng*, 20: 299–309.

WANG Y, WIG T, TANG J and HALLBERG LM (2003), 'Radio frequency sterilization of packaged foods', *J Food Sci*, 68(2): 539–544.

WONG E, LINTON RH and GERRARD DE (1998), 'Reduction of *Escherichia coli* and *Salmonella senftenberg* on pork skin and pork muscle using ultraviolet light', *Food Microbiol*, 15: 415–423.

WRIGLEY DM and LLORCA HG (1992), 'Decrease of *Salmonella typhimurium* in skim milk and egg by heat and ultrasonic wave treatment', *J Food Protect*, 55(9): 678–680.

ZENKER M, HEINZ V and KNORR D (2003), 'Application of ultrasound-assisted thermal processing for preservation and quality retention of liquid foods', *J Food Protect*, 66(9): 1642–1649.

19

Hazard Analysis Critical Control Point and other food safety systems in milk processing

S. C. Murphy, Cornell University, USA

Abstract: Hazard Analysis Critical Control Point (HACCP) principles are used throughout the food industry as a means to identify and control potential food safety hazards. Implemented HACCP systems have become important to dairy processors as a means to improve the safety of their products as well as for meeting customer demands and regulatory requirements. HACCP systems are based on seven established principles, or steps, and supporting prerequisite programs that include good manufacturing practices and sanitary standard operating procedures. The principles include performing a step by step hazard analysis based on a product flow diagram; determining critical control points and critical limits for controlling the identified hazards; establishing monitoring procedures for the critical control points and corrective action procedures for when monitoring determines that critical limits have not been met; developing HACCP plan verification procedures; and maintaining all appropriate records. The application of HACCP principles and related food safety systems to pasteurized fluid milk operations, with reference to regulatory based model systems, will be discussed.

Key words: HACCP, pasteurized milk, food safety.

19.1 Introduction

Application of Hazard Analysis Critical Control Point (HACCP) principles has become standard in the food industry as a means to identify and control potential hazards (biological, physical and chemical) associated with a food that could

result in human illness or injury. HACCP systems have been applied to nearly all foods, including fluid milk and dairy products, and are recognized throughout the world. While primarily practiced at the industry level, HACCP principles are becoming part of regulatory systems. This chapter will provide background on the history and development of the HACCP concept and will describe the application of HACCP principles to pasteurized fluid milk operations, with reference to regulatory based model systems such as the program developed for Grade 'A' Dairy products under the US National Conference on Interstate Milk Shipments (NCIMS) and the Food and Drug Administration (FDA). In addition to HACCP, other food safety and quality systems will be briefly discussed.

19.2 Background to the Hazard Analysis Critical Control Point (HACCP) concept

Applying HACCP principles in food manufacturing requires a systematic evaluation of the food and the process to identify potential hazards that may be associated with the food (the hazard analyses) and the development of means and measures that ensure that the identified hazards are controlled such that illness or harm is unlikely (the critical control points). HACCP principles are only effective if they are supported by well-documented prerequisite programs (PPs) that form the foundation of the HACCP system. While PPs are managed separately, they play an integral part in the hazard analysis and determination of critical control points and provide the basic operating and environmental conditions and procedures that are required for the production of safe food (NACMCF, 1998). Many of the PPs that support HACCP systems are based on regulatory requirements and guidelines such as those found in the current Good Manufacturing Practices (US-CFR 2007a, 21 CFR Part 110) and the CODEX General Principles of Food Hygiene (CAC, 2003). The HACCP system must take into account the impact of all aspects of the process from raw materials and ingredients to the distribution of the final packaged product.

Originally developed in the US for the National Aeronautics and Space Administration (NASA) in the early 1960s, HACCP principles were first presented to the public in 1971. The National Academy of Sciences (NAS), the National Advisory Committee on the Microbiological Criteria for Foods (NACMCF) and the Codex Alimentarius Committee on Food Hygiene of the Codex Alimentarius Commission (CAC) played significant roles in the development of HACCP principles and application guidelines that are currently used for HACCP program development (Scott and Stevenson, 2006). Table 19.1 presents a timeline for documented activities in the evolution of the HACCP principles that are in practice today. In the US, the NACMCF document titled 'Hazard analysis and critical control point principles and application guidelines' (NACMCF, 1998) serves as the model guidance document for applying HACCP principles to foods. A similar guideline published by the CAC and annexed into the Recommended Code of Practice (RCP), 'Recommended international code

Table 19.1 Timeline for documented activities in the development of HACCP system criteria

Year(s)	HACCP development event
1959	NASA commissioned the Pillsbury Company to manufacture food products safe for space travel. Pillsbury worked with US Army Natick Laboratories to develop the initial HACCP concept.
1971	The HACCP concept was first introduced to the public at the 1971 National Conference on Food Protection. It was based on three principles (hazard identification and assessment; determination of critical control points; establishment of monitoring systems).
1970s	Interest in HACCP rose (FDA began training inspectors and conducted select HACCP based inspections, HACCP was a topic of several conferences) and then fell. There was limited use of HACCP in the industry due to time and experience required. Titles 21 CFR Part 113 and 21 CFR 114 were developed in a manner reflecting HACCP.
1985	Report on the microbiological criteria for foods issued by the Food Protection Committee of the National Academy of Sciences (NAS) gave a strong endorsement of HACCP.
1988	In response to NAS recommendations, the National Advisory Committee on the Microbiological Criteria for Foods (NACMCF) was appointed by the US Secretary of Agriculture to serve as an expert advisory panel to federal food safety agencies. A HACCP working group was appointed to make recommendations.
1989	The NACMCF adopted a document outlining seven HACCP principles and a systematic approach for the application of HACCP.
1992	First revision of the NACMCF document adopted and published in the *International Journal of Food Microbiology*, 16: 1–23. Compared to a draft document prepared by the Codex Alimentarius Commission (CAC) Committee on Food Hygiene.
1993	CAC adopted *Guidelines for the Application of the HACCP System*.
1997	Second revision of the NACMCF document prepared and published subsequently in 1998 (*Journal of Food Protection*, 61: 1246–1249).
1997	CAC adopted *Guidelines for the Application of the HACCP System*, ALINORM 97/13A Appendix II. Annexed into CAC/RCP 1-1969, Rev 3-1997, *Recommended International Code of Practice – General Principles of Food Hygiene*.
2003	*HACCP System and Guidelines for its Application* annexed in CAC/RCP 1-1969; Rev 4-2003 was updated.

Source: adapted from Scott and Stevenson (2006).

of practice – General principles of food hygiene', CAC/RPC 1-1969, Rev. 4-2003 (CAC, 2003), is used by many other countries. Both documents provide sufficient background and cover the definitions, the preliminary steps (Table 19.2) and the seven principles (Table 19.3) that have become standardized in HACCP training and system development.

While the adoption of HACCP principles had a slow start, they have since been applied to many food commodities and processes and have become part of the regulatory requirements for certain foods. Under the US Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS), 'Pathogen

Table 19.2 Preliminary steps for HACCP system development as described in the NACMCF and CODEX documents

	NACMCF (NACMCF, 1998)	CODEX (CAC, 2003)
Step 1	Assemble the HACCP team	Assemble the HACCP team
Step 2	Describe the food and its distribution	Describe the product
Step 3	Describe the intended use and consumers	Identify intended use
Step 4	Develop a flow diagram that describes the process	Construct the flow diagram
Step 5	Verify the flow diagram	On-site confirmation of flow diagram

Table 19.3 The seven principles of HACCP and definitions of related terms

	HACCP principle (NACMCF, 1998; CAC, 2003)	Definition of italicized word(s) (NACMCF, 1998)
P-1	Conduct a *hazard analysis* (list all hazards, consider control measures)	The process of collecting and evaluating information on hazards associated with a food to decide which are significant and must be addressed in the HACCP plan
P-2	Determine *critical control points*	A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or to reduce it to an acceptable level
P-3	Establish *critical limits*	A maximum or minimum value to which a biological, chemical, or physical parameter must be controlled at a CCP to prevent, eliminate or reduce to an acceptable level the occurrence of a food safety hazard
P-4	Establish *monitoring* procedures for each CCP	To conduct a planned sequence of observations to assess whether a CCP is under control and to produce an accurate record for future use in verification
P-5	Establish *corrective actions*	Procedures followed when a deviation occurs (when a critical limit is not met at a CCP)
P-6	Establish *verification* procedures	Activities other than monitoring that determine the validity of the HACCP plan and that the system is operating according to the plan
P-7	Establish *record-keeping* and documentation procedures	

reduction – HACCP systems' were mandated under the Code of Federal Regulations as of 1996 for federally inspected meat and poultry operations (US-CFR, 2008, 9 CFR Part 417). Under the US Food and Drug Administration (FDA), processors of fish and fishery products (US-CFR, 2007c, 21 CFR Part 123) and manufacturers of 100% juice (US-CFR, 2007b, 21 CFR Part 120) are required to have HACCP systems in place. While not specifically required for most foods, the Canadian Food Inspection Agency's (CFIA) Food Safety Enhancement Program (FSEP) encourages and supports the development, implementation and maintenance of HACCP systems and has a procedure in place for HACCP recognition in federally registered establishments (CFIA, 2007). For federally registered meat processing plants in Canada, HACCP based systems under the FSEP are required (FDC, 2009). Federally registered fish processing plants in Canada are required to develop and implement HACCP based systems and quality control programs under the CFIA 'Quality Management Program' (CFIA, 2008). In the European Union (EU), Directive 93/43, which became effective in 1995, mandates best practices for food hygiene and safety with the application of HACCP based systems (EEC, 1993). The New Zealand Food Safety Authority has incorporated HACCP principles into codes of practice for meat (NZFSA, 2004), seafood (NZFSA, 1997) and dairy (NZFSA, 2003a, 2003b). Undoubtedly, HACCP principles play a role in other regulatory systems as well.

While not specifically defined as such, the dairy industry has operated under HACCP principles since the adoption of pasteurization. Hazards were identified in raw milk and pasteurization was developed, validated, monitored and verified as a critical control point (IOM/NRC, 2003). In recent years, formalized HACCP programs have become commonplace for dairy operations, as a means to ensure product safety and in many cases to satisfy customer and/or regulatory requirements. Although HACCP is not required by the regulatory agencies in the US, under the Grade 'A' Pasteurized Milk Ordinance (PMO), Grade 'A' dairy plants can now be regulated under the 'HACCP Alternative' instead of the traditional inspection/rating based system (FDA, 2007). The concept originated in 1997 when a committee was appointed by the National Conference on Interstate Milk Shipments (NCIMS) to address how a voluntary HACCP system could be implemented, evaluated, monitored and enforced as an alternative to the traditional inspection and rating (FDA, 2000, 2008b). In 1999, the committee's proposal to investigate a HACCP alternative was approved by the NCIMS. The general guidelines for the development of the PMO-HACCP Alternative were that it:

- be developed based on HACCP principles as defined by the NACMCF and be consistent with FDA HACCP recommendations (e.g., Juice HACCP regulations);
- continued to assure at least the same level of milk safety provided by the traditional inspection/rating/check-rating system; and
- continued to provide uniformity and reciprocity between states as did the traditional inspection/rating/check-rating system.

Using a select number of volunteer dairy plants, the proposed HACCP based regulatory system was investigated and modified during a two-phase pilot program. As a result, a HACCP based inspection system was proposed to the 2003 NCIMS conference, where it was adopted and subsequently included in the 2003 PMO under Appendix K. The NCIMS-HACCP alternative provides a model system for dairy HACCP programs based on the NACMCF principles that will be referenced throughout this chapter along with other model regulatory guidelines and references.

19.2.1   HACCP plan vs. HACCP system

The NACMCF and CODEX HACCP documents stress specific steps, principles and definitions that provide uniformity in the development of HACCP based programs. The NACMCF defines the HACCP plan as 'the written document that is based on the principles of HACCP and that delineates the procedures to be followed' whereas CODEX defines it as 'a document prepared in accordance with the principles of HACCP to ensure control of hazards'. This includes the development and documentation of the seven principles of HACCP (Table 19.3) including the hazard analysis and the determination of critical control points. A HACCP system is defined by NACMCF as 'the result of the implementation of the HACCP plan(s)', thus it encompasses the HACCP plans for all products in the system; the preliminary steps (Table 19.2); and the prerequisite, regulatory and other programs that support the HACCP plan(s).

19.3   Hazard Analysis Critical Control Point (HACCP) in milk processing

19.3.1   Initial steps

Adopting and implementing a HACCP based system can be a monumental task. Initially when developing a HACCP system, companies should determine their level of commitment, define their objectives and then establish goals and directions to meet these objectives. Commitment must come from the top management and be instilled throughout the workforce. While the primary objective of developing a HACCP system should be to provide the safest product possible, other objectives often include meeting specific customer and/or regulatory requirements. When establishing goals, companies need to determine what is practical and achievable based on the available workforce and time and capital commitments, all within a goal of ensuring that the HACCP system will work. Obtaining sufficient background in order to understand how HACCP fits into a plant's current programs and regulatory requirements is essential, which makes effective research and training among the most critical preliminary steps.

Table 19.2 lists the preliminary steps or tasks that lay the groundwork for the development of a HACCP plan as outlined by the NACMCF (1998) and CAC (2003) documents. The first step is to create the HACCP team, members of

which will be responsible for the development, implementation and maintenance of the HACCP system. Members of the HACCP team may be involved in the initial development of the objectives and goals listed above. Where practical, the team should include representatives of all functional areas including production, sanitation, quality control/assurance, maintenance, engineering and perhaps even marketing. If upper management decides not to be an active part of the team, support from the top should be clear. While those with supervisory or decision-making positions are often included, operational employees, such as pasteurizer operators, might also provide valuable input to the team, as they are more intimately involved in the process. It may be advantageous to recruit outside consultants or others with expertise (e.g., a university microbiologist) to fill gaps in the knowledge base. Forming a multidisciplinary team may be easy for larger dairy operations, but small plants may have a limited pool of potential team members; in some cases the 'team' may include nearly all employees.

In order for the HACCP team to function properly, it is important that all members have a clear understanding of their roles and responsibilities; documenting team members, their expertise (e.g., training) and their specific assigned activities are helpful (IDFA, 2002). Although differing responsibilities may suggest that some team members do not need to be involved in certain functions, it is important that all team members be kept informed of all activities and changes in the system. Often one person in a dairy operation is assigned the bulk of the oversight of implementation of the HACCP system (e.g., quality assurance). It is essential that others have sufficient knowledge of the system and that backup or contingency plans exist in case this person leaves the operation. Ideally all team members should be formally trained in HACCP. As the program progresses, appropriate training in the procedures required to implement the HACCP system should be provided for all employees so they understand the importance of their positions and responsibilities, regardless of whether they are on the team. For the NCIMS-HACCP program, formal training in the HACCP core curriculum, which includes basic HACCP principles as presented in the NACMCF document and an orientation to the requirements of the NCIMS-HACCP program, or 'equivalent experience', is required for persons who develop the hazard analysis and determine critical control points; who develop, validate and modify the HACCP plan; and who perform HACCP plan records review (FDA, 2007).

The second and third preliminary steps in developing a HACCP plan are to describe the food and its distribution (step 2) and describe the intended use of the food and targeted consumers (step 3). Standardized forms that ask for a basic product description, storage and distribution and intended use have been developed and are used to accomplish these two tasks. Table 19.4 provides an example of a more detailed form that might be used for pasteurized fluid milk. This form provides specific information that is important in determining the potential safety concerns associated with a food that would be significant in the hazard analysis. Product description forms need to be created for each product in

Table 19.4 Example of a product description form for pasteurized fluid milk

Product Description Form	
1. Product name(s)	Pasteurized homogenized milk (includes reduced fat, low fat and non-fat); Vitamins A and D added.
2. Important food safety characteristics (a_w, pH, processing, etc.)	Pasteurized but perishable; fits definition of a 'Potentially Hazardous Food' that requires refrigeration (high a_w, neutral pH, no preservatives added). Supports a variety of microorganisms.
3. Ingredient list	Milk, vitamins A and D.
4. Packaging used	Quart and pint paperboard (from supplier); gallon and half-gallon HDPE blow-molded plastic (manufactured in-house); filled on sanitized equipment.
5. Labeling requirements/instructions	'Store refrigerated at 40°F (4.4°C) or less'; 'Best if used within 7 days after opening or within 3 days post sell-by date, whichever comes first.'
6. Shelf-life	'Sell-by date' listed at 16 days post packaging. Should last to 18 days under proper refrigeration.
7. Storage and distribution handling	Stored refrigerated at 40°F (4.4°C) or less. Distributed refrigerated at 40°F (4.4°C) or less.
8. Distribution area and outlets	Shipped from plant to warehouse to retail or directly to retail outlets in the north-east US.
9. Intended consumers	All people of all ages including those in potential high risk groups (e.g., young, elderly, immuno-compromised).
10. Intended use	Ready-to-serve beverage; may be combined with other foods (cereal, coffee) or used as an ingredient (cooking, baking).

Company name and address: Phone:
                                                       Fax:
                                                       e-mail:

Version/date:
Approval signature: _____   Date signed: _____

Sources: adapted from the Food Safety Enhancement Program (CFIA, 2007) and IDFA's HACCP Plant Manual, 2002 edition (IDFA, 2002).

the HACCP system that is significantly different to warrant its own flow diagram, hazard analysis and/or HACCP plan.

The fourth and fifth preliminary steps, developing and verifying a flow diagram that describes the process, provide the basic road map for the product's hazard analysis (principle 1). A flow diagram should provide a clear, simple, sequential step-by-step description of the process. It should include all steps under the control of the facility from receiving raw materials and ingredients to shipping the packaged product (NACMCF, 1998). All inputs and edible outputs of the process should be shown (NZFSA, 2003b). A simple block-style diagram is commonly used for a flow diagram. There is no need to include details that would be in a plant blueprint, such as pumps, pipelines, valve clusters or sensors, but sufficient detail must be included to accurately describe the process. All steps where the product is treated or handled in a manner that might alter or influence the product such that hazards might be introduced, enhanced or controlled should be included. For products that have similar attributes and are

handled and processed in a similar manner, flow diagrams can often be combined (e.g., one flow diagram can be used for non-fat milk, low-fat milk and whole milk; a separate flow diagram might be created for chocolate milk).

Figure 19.1 provides an example of a basic flow diagram for a HTST fluid milk processing system. In this example, the separator is located in a split raw-regeneration section and the milk is packaged in either paperboard or HDPE jugs. Pasteurization is sometimes included in the flow diagram as a single step. It is recommended that the process be broken down into the specific sections, as each

Fig. 19.1   Example of a flow diagram for pasteurized white fluid milk products with in-line standardization in a split regeneration section.

should be considered in the hazard analysis. While not specifically included in the flow, the steam/water used in the heating step and the chill water used in the cooling step are noted in this diagram because they may introduce potential hazards (e.g., cross-contamination from leaky plates). Multiple packaging systems may be combined into one step in the flow diagram, although for this example, the paperboard and the HDPE fillers were displayed separately based on differences in source packaging materials (i.e., one purchased, one manufactured in-house). Other items that might be included in a flow diagram for conventionally pasteurized fluid milk products include the use of rework, the addition of ingredients (e.g., cocoa and sugar for chocolate milk) and blending operations and the use of air-blows for line clearing. For extended shelf-life or shelf-stable products steam injection and vacuum treatment, package treatment (e.g., hydrogen peroxide) and sanitizer rinses should be considered. Flow diagrams must be verified (step 5), initially and on a scheduled basis thereafter and/or after changes are made in the process. Verification is performed by on-site inspections of all stages and if possible at all critical times of the process and should include discussions with all team members and floor processing employees.

   Under the CFIA Food Safety Enhancement Program (CFIA, 2007), a plant schematic is also required. This diagram should provide a basic layout of the plant showing the receiving, storage, handling and shipping areas for raw milk, ingredients and other materials; the general process flow including packaging and handling of the finished product; traffic patterns of employees handling raw milk or product and employees handling pasteurized milk or product; and the location of break rooms, locker rooms, offices and rest rooms. The schematic serves as a reference for determining potential areas for cross-contamination (e.g., raw milk to finished product, allergen-containing ingredients/products with non-allergen-containing ingredients/products) and should be used to prevent high-risk activities and/or to redesign product and ingredient flow, handling and storage activities and traffic patterns to reduce or eliminate high-risk situations.

19.3.2   Prerequisite programs

Prerequisite to conducting a hazard analysis and developing and implementing a HACCP plan, dairy companies need to ensure that they have in place effective programs designed to provide the basic environmental and operating conditions required to manufacture safe, wholesome food (NACMCF, 1998). Prerequisite programs (PPs) are considered in the hazard analysis when determining if a critical control point (CCP) is needed for an identified hazard. In many cases, the PP is sufficient to reduce the likelihood of a hazard, such that a CCP is not warranted. PPs are generally broad-based programs applied throughout the operation, while CCPs are most often specific points in processing where control measures can be applied. PPs are defined as 'developed, documented, and implemented procedures, including current Good Manufacturing Practices (cGMPs) that control operational conditions that serve as the foundation for

the HACCP plan' (IDFA, 2002; NACMCF, 1998). A CCP is 'a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or to reduce it to an acceptable level'. When PPs are in place, the focus of the HACCP plan becomes the hazards associated with the product or the process, which must be controlled, and not the manufacturing plant environment (Gombas, 2006). According to Bernard *et al.* (2006), the primary differences between PPs and CCPs are as follows:

- PPs most often address hazards and food safety issues indirectly.
- PPs are more general in scope and may be applied throughout the operation, and to multiple process lines.
- Failure to meet a PP requirement (non-conformity) seldom results in a food safety hazard or concern, while a deviation from a critical limit typically results in action against the product.

Examples of prerequisite program areas that would apply to all foods as well as fluid milk establishments include sanitary design and control of facilities and equipment; cleaning and sanitation of facilities and equipment; environmental monitoring; supplier control to ensure the safety of ingredients and packaging; written product specifications; allergen management; personal hygiene and health of employees; employee training; control of chemicals and toxic compounds; receiving, storage and shipping of raw materials and finished products; traceability and recall programs; and pest control (IDFA, 2002; NACMCF, 1998; Bernard *et al.*, 2006). Application of general regulatory or code of practice guidelines such as those described in cGMPs (US-CFR, 2007a, 21 CFR Part 110) and CODEX general principles of food hygiene (CAC, 2003), as well as specific guidelines for dairy such as those spelled out in the PMO (FDA, 2007) and other regulations, are the basis for many prerequisite programs. In order for PPs to be effective, they should be well documented with clearly written SOPs that are reviewed periodically and revised as needed (Bernard *et al.*, 2006). Employees responsible for implementation must be well trained and understand the importance of the procedures, of the documentation and of correcting non-conformities. In addition to prescribed monitoring procedures, PPs should include routine verification procedures, such as review of monitoring records for completeness (e.g., weekly) and effectiveness of the procedure (e.g., ATP swabs for cleaning efficiency).

Under the NCIMS-HACCP system, there are eight required prerequisite programs (Table 19.5). These PPs mirror the required sanitation standard operating procedures (SSOPs) outlined for the FDA's juice (US-CFR, 2007b; 21 CFR Part 120) and seafood (US-CFR, 2007c; 21 CFR Part 123) HACCP requirements. A brief written description or checklist is required for each PP. This written summary is used in the regulatory auditing process (to verify compliance), but also serves as a reference for the dairy plant to use in implementing the program and verifying that the program is followed as intended (e.g., for self-audits). The description should include a brief summary of the purpose and the procedures covered by the PP, including who performs the

Table 19.5   Required prerequisite programs under the NCIMS-HACCP program

Prerequisite program	Items that may be covered/monitored
1. Safety of water that comes into contact with milk product or product contact surfaces, including steam and ice	Water source supply; chill water, ice and steam; reclaimed water; backflow prevention; no cross-connections
2. Condition and cleanliness of product contact surfaces	Approved, cleanable, food contact surfaces; SOPs for cleaning and sanitation; chemical concentrations, temperatures, times and mechanical action; preventative maintenance programs
3. Prevention of cross-contamination from in-sanitary objects and/or practices to milk products, milk product contact surfaces, packaging, material and other food contact surfaces, including gloves, outer garments, etc. and from raw product to processed product	Employee hygiene; traffic flow and plant layout; separate equipment and utensils used for raw and pasteurized milk; condition and cleanliness of utensils and cleaning aids; environmental sanitation; waste management; pressure differentials in HTST units; no cross-connections
4. Maintenance of hand washing, hand sanitizing and toilet facilities	Facilities available where needed; supplied and maintained clean; hot water; hand-washing/sanitizing procedures
5. Protection of product, packaging, and product contact surfaces from adulteration with lubricants, fuel, pesticides, cleaning compounds, sanitizing agents, condensate and other chemical, physical and biological contaminants	Separate storage; protection of product (covers/caps), ingredients and packaging; proper drainage; use of food-grade lubricants; proper use of chemicals; proper ingredient addition (e.g., vitamins); shielded lighting; pesticide application; air-line filters; HTST plate maintenance
6. Proper labeling, storage and use of toxic compounds	Separate storage; original labels intact; working/temporary containers labeled; only approved chemicals used and according to instructions
7. Control of employee health, including exposure to high risk situations, that could result in the microbiological contamination of milk products, packaging, and product contact surfaces	Employee GMP training and knowledge of requirement to report illnesses; observations and signs of employee health; wound protection procedures
8. Pest exclusion from the milk plant	Presence/absence of pests; exclusion techniques; control of pest attractants and harborage; bait and trap; pesticides

Source: Appendix K of the Pasteurized Milk Ordinance (FDA, 2007) and Gombas (2006).

procedure and at what frequency; a listing of the monitoring procedure(s), including who monitors and at what frequency; a description of the records and their storage location; and an outline of corrective steps to be taken when monitoring indicates that the goals of the PP are not met (non-conformities or deficiencies) (FDA, 2007; IDFA, 2002). Details of the procedures used to implement the PP, such as would be written in an SOP (e.g., CIP procedure for a milk line), are generally not necessary for this summary, but may be referenced.

The practices and outcome of each PP must be monitored at a frequency that ensures that the specified objectives are met and that product safety is not jeopardized. Frequencies must be auditable; 'as required' or similar phrases are not auditable frequencies for monitoring (CFIA, 2007). For example, monitoring for cleaning and sanitizing should be done daily at the end of the performed tasks and should be verified before start-up of the next scheduled processing; monitoring of pest control might be done on a weekly basis. Where applicable, devices used to monitor (e.g., recording thermometers) must be properly calibrated. When monitoring reveals deficiencies or non-conformities in a PP, corrective procedures must be implemented and documented. If the goals of a PP are not consistently met, it may be that it needs to be modified. Verification that PPs are being implemented as planned would include reviewing monitoring records, periodic inspections and testing related to the PP (e.g., surface hygiene swabs for cleaning/sanitizing, allergen rinse tests). Records of monitoring, corrective procedures and verification procedures must be kept and be available for review. A similar write-up is required for PPs in the CFIA-FSEP program (CFIA, 2007), which uses a format similar to the HACCP plan summary that will be described later in this chapter. CFIA-FSEP PPs are comparable to the NCIMS-HACCP program and include premises (exterior, interior, sanitary facilities, water/steam and ice); transportation, receiving and storage; equipment (design, installation, maintenance and calibration); personnel (training, hygiene and health); sanitation and pest control; and recalls. Deficiencies in the implementation of PPs that cannot be corrected under the CFIA-FSEP guidance require both short-term and long-term action plans.

In the fluid milk industry other programs in addition to the required eight PPs listed in the NCIMS-HACCP program should be in place and are generally essential in supporting the hazard analysis and the HACCP plan in most dairy operations. These would include requirements for receiving and storing raw materials, including temperature requirements; storage and handling of finished product, including temperature requirements; drug residues in raw milk; supplier control, including performance and safety criteria, product specifications, certificates of analysis/certificates of guarantee and tracking records; allergen control where applicable (sometimes covered under prevention of cross-contamination or adulteration); vitamin fortification procedures; product specifications; handling and use of rework; personnel training; preventative maintenance programs; equipment maintenance and calibration; labeling; complaint documentation and investigation; food defense procedures; and recall and traceability (Bernard *et al.*, 2006; IDFA, 2002; NACMCF, 1998).

19.3.3   Hazard analysis and critical control point determination

A hazard analysis (HACCP principle 1) is the process of collecting and evaluating information on hazards potentially associated with a food (biological, physical, or chemical) to determine which are significant and must be addressed in the HACCP plan, e.g., controlled. A critical control point (CCP) is a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable limit (NACMCF, 1998). The hazard analysis is a logical process where each activity or step identified in the flow diagram is evaluated for the likelihood of a hazard being introduced, enhanced or controlled. When conducting a hazard analysis, biological, chemical and physical hazards must be considered at each step. Where potential hazards are identified, the next step is to determine the likelihood of occurrence and possible severity of the hazard. This is where PPs are often considered. An established PP may reduce the likelihood of a hazard such that a CCP is not needed, or a PP may be developed or modified to do so. If a hazard is deemed likely to occur and/or is severe, then it must have a CCP either at the point where it was identified or later in the process. Decision trees are available to help the HACCP team determine whether a hazard must be controlled at a CCP (IDFA, 2002; NACMCF, 1998); however, the general rule is that if the hazard is likely to cause illness or injury in the absence of its specific control, then a CCP is required. Determining if an identified hazard must be controlled by a CCP or can adequately be addressed by a PP is often debatable. Regardless, if a hazard is identified, there should be a procedure in place that eliminates, prevents or reduces it to an acceptable level. Common practice in HACCP development in the dairy and food industries is to minimize the number of CCPs to keep the system manageable. In fluid milk processing, pathogens in raw milk are always considered biological hazards reasonably likely to occur, and pasteurization is the CCP for this hazard. Other identified potential hazards may be controlled under PPs or CCPs depending on the plant's hazard analysis. Table 19.6 presents a hazard analysis worksheet commonly used to identify potential hazards at each step taken from the flow diagram and to determine if the identified hazards are likely and if a CCP is needed.

To keep the hazard analysis process practical, care should be taken to only consider hazards that have been shown through science, product composition and characteristics, processing and handling procedures, outbreak/illness data or experience to be associated with the food in question or with similar foods. Where applicable, risk assessment techniques can be used to determine the likelihood of a hazard. There is substantial information on the microbiological safety of fluid milk and other dairy products, much of which is outlined in other chapters of this book. Potential chemical hazards associated with raw and processed dairy products have been well documented, while the potential for physical hazards is dependent on product types and specific processes. For the NCIMS-HACCP program a hazards and controls guide has been developed to assist in the hazard analysis process (FDA, 2006a). Following are summaries of hazards described in this document and others that might be considered in fluid milk operations.

Table 19.6 Example of a hazard analysis worksheet

(1) Ingredient Processing Step	(2) Identify potential food safety hazards introduced, controlled or enhanced at this step.	(3) Are any potential food-safety hazards reasonably likely to occur (do they need to be addressed in the HACCP plan)? (YES/NO)	(4) Justify your decision for column 3 (WHY?)	(5) What control measure(s) can be applied to prevent, reduce, or eliminate the food safety hazards?	(6) Is this step a critical Control point? (YES/NO)
List steps from the flow diagram in order that they appear.	For each step, list identified potential hazards as: Biological Chemical Physical Leave blank if no hazards are identified. Only fill in columns 3–6 if a hazard is listed. Do not carry the hazard through subsequent steps unless it is further enhanced or controlled.	Answer 'YES' or 'NO' for each identified hazard.	If column (3) is 'YES,' provide rationale, based on science, regulation and/or history, why the hazard is likely and must be controlled in the HACCP plan. If column (3) is 'NO,' describe the prerequisite program or other activity that will reduce the likelihood of the hazard.	If column (3) is 'YES,' describe the control measure that will reduce, eliminate or reduce to an acceptable level the identified hazard. If column (3) is 'NO,' leave this column blank.	If column (3) is 'YES,' and this is the CCP for the identified hazard, answer 'YES.' If the CCP is elsewhere, answer 'NO.'

Sources: adapted from FDA (2007) and Scott and Stevenson (2006).

Biological hazards

In fluid milk processing, vegetative pathogens associated with raw milk are considered biological hazards that are reasonably likely to occur. Those most commonly identified include *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni* and enterohemorrhagic *E. coli*. While the instances of *Coxiella burnetii*, *Mycobacterium bovis/tuberculosis* and *Brucella* spp. have been reduced in many areas of the world, they should not be overlooked, as there have been recent associations of these organisms with raw milk and raw milk products (CDC, 2005; Kim *et al.*, 2005; Mendez Martinez *et al.*, 2002). The CCP for vegetative pathogens in raw milk is pasteurization, specifically the heat and hold step. Recontamination of milk after pasteurization should also be considered in the hazard analysis, but this is normally controlled under PPs that cover cleaning and sanitization, prevention of cross-contamination and employee health and hygiene, thus reducing the likelihood of occurrence. Establishing a CCP to prevent recontamination is not practical as there are no specific points where control could be applied; the listed PPs cover the whole system. During milk storage, on both the raw and the pasteurized side, there is potential for further growth of pathogens and possible toxin production (e.g., *Staphylococcus aureus* or *Bacillus cereus* enterotoxins). This can be controlled by proper refrigeration at storage; some have considered storage temperatures as CCPs, but this might also be covered under a PP for storage, handling and temperature control and monitoring.

Chemical hazards

Chemical hazards associated with raw and pasteurized milk that might be identified in a hazard analysis include animal drug residues, pesticides, mycotoxins, cleaning and sanitizing chemicals, allergens, and ingredients that might be toxic when added in excess (e.g., vitamins A and D). Antibiotics commonly used in treating dairy animals may present a hazard to allergic or sensitive individuals, although documented cases of this are rare. Chapter 5 discusses the issues of residues in milk. Testing programs that screen tanker-loads of milk at receipt for commonly used drugs (e.g., beta-lactam antibiotics), such as required under Appendix N of the PMO, are generally effective in reducing the likelihood of contaminated milk making it through processing to the consumer. Summaries of the US National Drug Data Base (Anon., 2008), which tabulates information on the results of drug residue testing programs, have shown that the incidence of antibiotic-contaminated milk in the US is relatively low. In the annual report ending September 2007, only 0.032% of over three million tanker-loads of raw milk tested positive. Only two out of 43,851 samples (0.005%) of pasteurized milk products analyzed were positive. A majority of the test results were for beta-lactam antibiotics; other drugs tested for in random samplings included sulfonamides (five out of 47,915 positive), tetracyclines (two out of 11,874 positive), aminoglycosides (two out of 640 positive), macrolides (one out of 860 positive) and enrofloxacin (none out of 1,579 positive). While a CCP for drug residues might be considered (e.g., screening tankers), addressing this under a

PP may be more appropriate based on the low frequency and the low risk. In the NCIMS-HACCP program, plants are required to be in compliance with the drug residue testing program as specified in Appendix N of the PMO. While generally handled separately from the HACCP plan, effective monitoring for drug residues as prescribed in Appendix N is considered a critical element for the plant's regulatory listing and continued operation under the Grade 'A' program. Within the program, plants are encouraged to screen for other residues deemed pertinent.

Pesticide residues are generally not considered significant, based on preventative regulatory programs and compliance in the US and other countries, and may not even be identified in the hazard analysis. These should be considered if testing or other information suggests that levels may exceed acceptable limits. Mycotoxins associated with contaminated cattle feed are typically not a concern in most areas, but might be identified in the hazard analysis if there is reason to do so, such as a wet growing season or results of feed tests. Screening milk, specifically for aflatoxin M_1, might be used as a control mechanism, although in areas where climate conditions favor mold growth and mycotoxin production, control should ideally be at the farm level (e.g., feed testing). Contamination with cleaning and sanitizing chemicals is most often covered under cleaning and sanitizing and other PPs. Procedures should ensure that chemicals are labeled correctly and used at proper concentrations, that solutions are rinsed and drained appropriately, and that there is adequate separation or breaks between product lines/vessels and cleaning chemical lines and vessels during processing.

Allergens need to be considered in the hazard analysis only if the dairy plant processes or handles non-dairy foods or ingredients that might be considered potential hazards. The eggs in eggnog would be considered a potential allergen for other milk products. For milk plants that also process soy beverages, soy would be a potential allergen in milk, and milk would be a potential allergen in the soy products. Many fluid dairy operations also process juice using much of the same equipment used for milk. In these operations, milk allergen would be considered the potential hazard for the juice products. If a fluid milk operation also processes ice-cream, nuts and other ingredients that might be potential allergens should be considered. For preventing allergen contamination, control often falls under one or more broad-based PPs; in most cases there are no specific points or steps where control can be easily applied to ensure that the allergen hazard was unlikely. Potential allergens can be addressed in a standalone 'allergen PP' and/or under programs for cleaning and sanitizing, prevention of adulteration and/or possibly prevention of cross-contamination. Included in allergen management programs would be separating allergen from non-allergen ingredients, products and packaging during storage and handling; scheduling process runs such that allergen-containing products are processed after the non-allergen products and that non-allergen products are run only after a complete, validated wash of the entire system; controlling the use of rework; and ensuring proper labeling and packaging of products (Gombas, 2006; IDFA, 2002). While many justify allergen control under PPs, where common

equipment is used, process order (e.g., processing non-allergen products only after a complete, validated wash procedure after allergen-containing products) may be considered a CCP.

Vitamins A and/or D should be considered in the hazard analysis for plants fortifying milk products, as over-fortification could result in toxic levels (Jacobus *et al.*, 1992). The target level for vitamins A and D in milk manufactured in the US is 2000 IU/quart and 400 IU/quart, respectively. The US-FDA currently considers levels in excess of 6000 IU/quart vitamin A and 800 IU/quart vitamin D to be potential health concerns (Nichols, 1992). Vitamin fortification might be controlled under a CCP or a PP, depending on a firm's hazard analysis. Preventing over-fortification is accomplished by careful monitoring of vitamin concentrate addition, proper measurement of pump feed rates, and determining whether the volume of concentrate used per product batch is in relative agreement with the theoretical value required to achieve the desired fortification level.

Physical hazards
Extraneous material (e.g., straw, dirt or wood) may be present in raw milk due to milking unclean cows or from farm environmental sources. Most milk is filtered at the farm and at the plant, thus extraneous material is often not considered in the hazard analysis. Other physical contaminants that might be considered in dairy operations include glass from bottles or unshielded lighting; plastic and rubber from equipment (e.g., gaskets) or packaging; and metal from equipment parts or wear. Whether these are identified in the hazard analysis would depend on the specific operation. Plants that bottle in glass packaging should in most cases consider glass fragments a likely hazard that should be handled as a CCP. Metal as a hazard in most fluid processing systems is generally considered unlikely, although consideration should be given if there is equipment with metal-to-metal moving parts. Preventative maintenance programs are generally effective in reducing the likelihood of metal and other equipment-related physical hazards. According to the US-FDA Compliance Policy Guide, Section 555.425, 'Foods – Adulteration involving hard or sharp foreign objects' (FDA, 2005), objects 7 mm to 25 mm in length would be considered physical hazards when in ready-to-eat foods served without preparation. Objects of less than 7 mm would be considered hazards for high-risk groups (infants, surgery patients, and the elderly). Control for physical hazards might include filtration at specific points in processing, metal detection, visual inspection and observations (e.g., in glass bottling operations or for equipment integrity) and preventative maintenance to prevent equipment wear.

Table 19.7 presents an alternative hazard analysis worksheet that covers the raw milk receiving and storage and the pasteurization heat and hold step identified as a CCP. This example shows where potential biological, chemical and physical hazards are identified and provides the justification for whether the hazards are significant, if they are adequately controlled by a PP or if a CCP is warranted. In this example the PPs that reduce the likelihood of a hazard are clearly defined.

Table 19.7 Example of a hazard analysis for the raw milk receiving and storage steps and the pasteurization heat and hold step using an alternative worksheet

(1) Per product flow diagram, list each ingredient or processing step	(2) Identify potential food safety hazards introduced, controlled or enhanced at this step: Biological Chemical Physical Proceed to column 3 only if a hazard is identified.	(3) If potential hazards are listed associated with this step, are they severe and reasonably likely to occur? (YES/NO)	(4) Justify the decision in column (3) and: • If 'NO,' explain why hazard was listed then identify the documented PPs, SSOPs or other procedure that will control or reduce the likelihood of the hazard. Consider if a PP/SSOP can be modified or newly established to control the hazard? • If 'YES,' explain why the hazard is listed as 'reasonably likely' and identify the control measure/step(s) required to prevent, eliminate or reduce the likelihood of the hazard.	(5) If column (3) is 'YES,' is this step a Critical Control Point (CCP)? (YES/NO). If 'NO' then identify where the CCP will be. (Other Notes)
Raw milk receiving	BIOLOGICAL: a. Pathogens (vegetative *Salmonella, Listeria, E. coli*, etc.) introduced.	a. YES	a. Numerous studies and historical data document that pathogens have been associated with raw milk. Further contamination during receiving and unloading is possible. For presence of pathogens, control point is pasteurization.	NO Controlled by pasteurization heating at a later step.
	b. Pathogen toxin development due to microbial growth in raw milk (introduced or enhanced) on the farm or in transit (e.g., *Staphylococcus* toxin)	b. NO	b. Growth of *Staph.* and potential toxin development due to temperature abuse has been a rare occurrence on the dairy farm and in the dairy industry. Milk temperature is monitored at pick-up. PP # 9, *Receiving, Handling and Storage of Ingredients* will ensure that loads are received at <7°C; PP # 10, *Testing Requirements for Receiving Loads of Raw Milk* reduces likelihood of receiving loads of milk with high bacteria counts.	
	CHEMICAL: Beta-lactam drug residues introduced.	NO	Required Appendix N testing and documentation of all loads of milk received will prevent the likelihood of accepting beta-lactam contaminated milk. Appendix N testing is listed under the requirements of PP # 10, *Testing Requirements for Receiving Loads of Raw Milk*, though is performed as a PMO requirement and under FDA 2400 form guidelines.	
	PHYSICAL: Extraneous matter introduced.	NO	There is limited opportunity for contamination of milk with physical hazards. Raw milk is generally filtered at the farm and runs through a filter during the unloading process. Filters are changed daily.	

Table 19.7 *continued*

Raw milk storage	*BIOLOGICAL*: Potential pathogen growth and toxin development (e.g., *Staphylococcus* toxin) introduced/ enhanced.	NO	PP # 9, Growth of *Staph.* and potential toxin development due to temperature abuse has been a rare occurrence in the dairy industry. PP # 9, *Receiving, Handling and Storage of Ingredients*; will ensure proper cooling and limited storage of raw milk, making growth and toxin development unlikely.	
	CHEMICAL: Cleaning chemicals/ sanitizers introduced.	NO	PP # 2, *Condition & Cleanliness of Food Contact Surfaces* – SOP ensures that all raw milk tanks are properly rinsed of cleaning chemicals and thoroughly drained after sanitization.	
	PHYSICAL: None			
Other steps …				
Pasteurization heating and holding tube step	*BIOLOGICAL*: Pathogens (vegetative *Salmonella, Listeria, E. coli*, etc.) controlled.	YES	This is the only step that can be adequately controlled to ensure the destruction of potential pathogens that might occur in raw milk. Milk will be heated to a minimum of 161°F and continuously monitored by the recording chart and a visual check by the operator at the start and end of each product type (e.g., whole, 2%, 1% and skim)	YES (CCP 1)
	CHEMICAL: Steam additives used may contaminate milk if leaks in plates exist (introduced).	NO	Significant leakage of steam through plates is unlikely due to inspection/maintenance of HTST under PP # 3 *Prevention of Cross Contamination*. PP # 1 *Safety of the Water that Comes into Contact with a Food* ensures that the steam used for HTST is of acceptable quality and approved additives used.	
	PHYSICAL: None			

19.3.4   The HACCP plan

Once the hazard analysis is completed and hazards that are likely to occur are identified and CCPs are determined, critical limits for each CCP need to be set (principle 3), monitoring procedures must be developed (principle 4), corrective actions need to be planned (principle 5), verification procedures need to be established (principle 6) and an effective record-keeping program needs to be put in place (principle 7). These activities are generally documented on what is commonly referred to as the HACCP Plan Summary Sheet (Table 19.8).

All CCPs in a HACCP plan must have one or more control measures with one or more critical limits. Critical limits (CL) are maximum and/or minimum values to which a control measure is set in order to prevent, eliminate, or reduce to an acceptable level the occurrence of the identified food safety hazard that must be controlled at a CCP (NACMCF, 1998). CLs are generally based on accomplishing at least one criterion, such as a 5-log reduction of a target pathogen, detecting a drug at 5 ppb level or removal of physical hazards greater than 7 mm. CLs must be science based and validated by research, including literature reviews and/or challenge studies (Scott and Stevenson, 2006). In the food industry, many CLs are set by regulatory policy. For the destruction of vegetative pathogens in raw milk, the CLs for pasteurization are based on minimum temperature and time combinations (e.g., minimum temperature of 72ºC held for a minimum time of 15 seconds). These have been established by regulatory agencies based on science and years of experience and are designed to inactivate the most heat-resistant non-spore-forming pertinent microorganism, i.e., *Coxiella burnetii* (FDA, 2007). In vat pasteurization, air-space temperature would also be a CL. In most fluid milk operations, the CLs for the pasteurization CCP are most often set at the regulatory minimum. Dairy plants may choose to establish 'operating limits' that provide a margin of safety over and above the set CLs. When monitoring indicates that a process exceeds an operating limit and is drifting toward a CL, adjustments can be made to prevent a deviation from occurring. Most fluid milk plants pasteurize milk well above the minimum temperature and holding time CLs required for legal pasteurization in order to provide that margin of safety. Operating limits may also serve other purposes beyond providing for a safe product, such as increasing the viscosity of a stabilized cream dressing or denaturing milk proteins for yogurt production. Other examples of critical limits that might be used in a fluid milk operation include 'no broken glass observed' for a glass packaging line, and measured vitamin concentrate used for a production run is not over a specified percentage of the calculated theoretical amount needed for the total amount of milk produced (e.g., a CL maximum of 20% over theoretical).

Monitoring of CCPs is performed to ensure that CLs are not exceeded, to assess whether the CCP is under control. If CLs are exceeded, a deviation from the plan has occurred and corrective actions must be taken (NACMCF, 1998). Monitoring must be done at a frequency that ensures that the control measures are effective in providing a safe product. Continuous monitoring is ideal, but not always practical. For milk pasteurization, continuous temperature recording

Table 19.8 Example of the HACCP plan summary table for pasteurization CCP

Critical Control Point (CCP)	Hazard(s)	Critical limits	Monitoring				Corrective action(s)[a]	CCP verification[b]	Records
			What	How	Frequency	Who			
Milk and milk products pasteurization heat and hold step	Biological-vegetative pathogens (non-spore formers)	Temperature ≥ 72°C (161°F)[b]	Temperature at the exit of the holding tube	Temp. recorder chart Visual check	Continuous during operation After each product	Pasteurizer operator Operator	Manually divert flow of product Isolate the affected product Evaluate and determine disposition of the product (reprocess or disposal) Document all actions	**Record review:** Pasteurizer charts verified daily by supervisor. **Equipment function checks:** Operator performs required daily tests and records on the temperature charts. Authorized plant person (supervised by regulator when required) conducts checks listed in the *Milk Plant Equipment Test Report* (FDA FORM 2359b). **Seals:** Verify required regulatory seals daily, record on chart.	Pasteurizer charts Corrective action records CCP verification records, including equipment testing records, seal
		Flow rate in continuous flow pasteurizers with magnetic flow meter based timing systems (to ensure minimum residence/holding time ≥ 15 seconds in HTST holding tube) Note: Assuring that the minimum holding times are met in systems which use a sealed timing pump would be as CCP verification during required equipment calibration.	Flow rate in the holding tube in continuous flow pasteurizers with magnetic flow meter based timing systems	Flow recorder chart Visual check	Continuous during operation After each product	Pasteurizer operator Operator			

[a] A properly operating HTST pasteurization system will divert raw product to the constant-level tank when predetermined set points are not met.
[b] Every particle of milk or milk product is heated, in a properly designed, calibrated and operated pasteurizer, as specified in the current *Grade 'A' PMO*. Pressures in the regenerator of continuous-flow pasteurizers, as required in the holding tubes, across steam injectors, and within infusion chambers shall be addressed in the HACCP Plan and managed as CCP verification(s).
Source: adapted from FDA (2007), Milk and Milk Product Continuous-Flow HTST Pasteurization – CCP Model HACCP Plan Summary.

charts are used to monitor the temperature at the end of the HTST holding tube or in agitated tanks for vat or batch pasteurization. Air space temperature in vat pasteurization could also be monitored, but is normally written on the milk temperature recorder at the start and end of the hold time. Although temperature is continuously monitored for pasteurization, monitoring temperature should also include a visual inspection of the recording chart on a preset periodic basis (e.g., visual inspection after each product type or every hour by the operator). For control of physical hazards in a glass line, monitoring would be visual observations either at set periods or possibly 'continuous' if an operator is always present. Continuous monitoring is not practical for many control measures. For control of physical hazards using an in-line screen, monitoring may simply be a handwritten record of a visual inspection of the screen being intact and in place at the beginning and end of a processing run. Monitoring vitamin fortification levels might include ensuring that the vitamin delivery system (e.g., feed pump) is running at start-up and, at the end of a process run, determining the amount of vitamin concentrate used, and calculating the percentage of theoretical based on the amount of milk processed. If batch fortification procedures are used, the amount of vitamin concentrate and the amount of milk processed would be recorded and compared to the calculated theoretical value. If vitamin levels were found to be significantly below the target, this would not be a deviation, but it should be corrected to comply with product labeling requirements.

While a minimum holding time is often listed as a critical limit for milk pasteurization, holding time is not actually monitored in HTST systems. In magnetic flow meter based timing systems, flow rate can be monitored using the recording chart with the system set for a maximum allowable flow rate to ensure the minimum holding time in the system's holding tube (e.g., flow rate and the low flow/loss of signal and high flow alarm settings). In systems using sealed, one-speed timing pumps, flow rate is generally not monitored, but flow should be locked at one speed. The actual holding time for both systems is determined by verification procedures (e.g., salt test and can fills) performed on a scheduled basis (e.g., quarterly) or when a system failure occurs. In vat or batch pasteurization, holding time can easily be monitored. In the HACCP plan summary sheet, what is monitored (e.g., temperature), how it is monitored (e.g., recording thermometer), the frequency at which it is monitored (e.g., continuously or at specified periods) and who is responsible for monitoring (e.g., the pasteurizer operator) are all documented for each CL for each CCP (Table 19.8).

When a CL is exceeded at a CCP, a deviation from the plan has occurred and corrective actions must be taken and documented. In the HACCP plan summary sheet, a listing of corrective procedures should be included that outline the plan of action. The corrective action plan may include predetermined activities that address specific causes of deviations or may be more generic in nature. At minimum, the plan should ensure that no potentially unsafe milk enters commerce; if it has already entered commerce, then it should be removed from the market (e.g., recalled) and the cause of the deviation should be corrected.

Under the NCIMS-HACCP program (FDA, 2007), when a specific corrective action plan is not predetermined for a deviation, then the milk plant shall do the following:

1. Identify, segregate and hold all affected milk.
2. Determine the acceptability of the affected milk for distribution or reprocessing if applicable.
3. Ensure that any milk deemed unsafe or adulterated as a result of the deviation does not enter commerce.
4. Take corrective actions to correct the cause of the deviation.
5. Perform timely validation to determine if modifications of the HACCP plan are needed to prevent recurrence of the deviation, and modify the HACCP plan as needed.
6. Fully document all deviation and corrective action activities.

In properly operating HTST pasteurization systems, when milk is diverted through the flow divert valve, this is not considered a deviation from a CL. If, however, the divert valve fails and milk below the CL temperature at the end of the holding tube is allowed to flow forward, this would be considered a deviation. Corrective action would be to manually divert the product and, at minimum, follow the steps outlined above (FDA, 2007). In a glass-filling operation, broken glass observed during filling would exceed the CL ('no broken glass observed'). Corrective action might involve removing a specified number of bottles before and after the breakage or from a designated distance from the shatter area, as well as a thorough clean-up of the filler and surrounding area.

To ensure that the HACCP plan is working, verification procedures (principle 6) must be in place. Verification includes those activities other than monitoring that determine the validity of the HACCP plan and that the system is operating accordingly (NACMCF, 1998), in other words that the plan is being implemented as written. Verification procedures must include review of the HACCP plan records to determine that all monitoring is being performed and that CL and PP goals are being met. Activities such as calibration, equipment checks and periodic end-product testing are also considered as verification procedures. Under the NCIMS-HACCP program, required verification activities include:

1. Calibration and/or inspection of CCP monitoring instruments (e.g., thermometers, flow meters) or equipment or other devices that influence the CCP (e.g., timing pump and divert valve seals).
2. Review of records that document CCP monitoring to ensure completeness and to verify that values are within established CLs, and if not, that deviations are documented in a centralized log and that corrective action has been taken.
3. Review of records of corrective actions taken when a deviation occurs to ensure that appropriate procedures were followed and documentation is complete, including the handling of the affected product.
4. Review of records that document the calibration and inspection procedures (1).

While generally not warranted under an effective HACCP plan, periodic end-product or in-process testing and review of associated records may be included as verification activities. End-product testing is rarely used as a CCP and is not recommended in most cases. All verification record reviews should be performed and signed by a person who is appropriately trained and positioned (e.g. supervisor) and not the person who originally created the record (e.g., the operator). The reviews should be done at a frequency that reflects the importance of the record, generally within a short time after records were made (FDA, 2007). For example, CCP pasteurization charts used as CCP records should be reviewed daily, before product is released.

For the milk pasteurization CCP, daily calibration and equipment check verification activities include cross-referencing the recording chart with the HTST reference thermometer for both HTST and batch methods, determining the flow diversion valve cut-in and cut-out temperatures, and inspecting equipment seals for HTST pasteurization (e.g., seals set by regulatory agencies to prevent modification). Other verification activities include periodic tests (e.g., quarterly or semi-annually) that ensure proper calibration, settings and equipment operation, such as checking the accuracy of the indicating and recording thermometers against a reference thermometer, determining product hold time (e.g., salt test) and ensuring that flow diversion devices are operating properly. These verification procedures are part of the requirements for proper pasteurization as described in regulations (e.g., PMO). Specific testing procedures required for US plants in the Grade 'A' Milk program are outlined in form FDA 2359b, Milk plant equipment test report (FDA, 2006b).

Validation is an element of verification focused on collecting and evaluating scientific and technical information to determine whether the HACCP plan, when properly implemented, will effectively control the identified hazards (NACMCF, 1998). Initial validation requires ensuring that the hazard analysis and the established CCPs and CLs are scientifically sound and will effectively eliminate, prevent or reduce to an acceptable limit the identified hazards. This may be based on review of current literature, regulatory guidance and/or consultation with experts in the field. Validation of the HACCP system should be performed periodically (e.g., annually as required under the NCIMS-HACCP program) or whenever something in the process warrants revalidation. This may include changes in raw materials, formulations, processing methods, equipment or packaging systems; changes in distribution or target customers; increased positive results in end-product testing; increased consumer complaints; or the occurrence of unexplained system failures or increased CL deviations. Revalidation should also be performed as new hazard concerns are identified through research or product outbreaks. When *Listeria monocytogenes* emerged as a potential pathogen in milk, several studies were performed to revalidate the pasteurization process for this organism. In addition to internal review of a plant's HACCP system, it is recommended that periodic reviews be conducted by outside experts as part of the verification process. Plants may hire private third-party auditors, or they may be audited by specific customers to ensure compliance with their requirements.

Effective record-keeping procedures (principle 7) document that the HACCP system is in place and is working. Auditing of a HACCP system, by either private firms or regulatory agencies, centers on the review of HACCP documentation for completeness and accuracy. Records required or that could help facilitate the auditing process may include (IDFA, 2002; FDA, 2007):

1. Table of contents and centralized list of HACCP program records
2. Listing of the HACCP team and assigned responsibilities
3. Written PPs and associated monitoring records
4. Product description sheets, verified flow diagrams and hazard analysis worksheets for each product type
5. HACCP plan summary tables for each product type including a listing of all CCPs and their associated identified hazard(s) and CL(s); procedures for monitoring, corrective actions and verification; and a listing of specific records and documentation
6. CCP and CL monitoring and verification records
7. Deviation log and corrective action records
8. Document change log (whenever the HACCP system is updated)
9. Supporting documentation.

All records should be clearly identified, should include pertinent plant information, and should be signed and dated by the person responsible for the record. Where applicable, records reviewed in a verification procedure should be signed and dated by the verifier. Whenever information is changed or when a record is subjected to a scheduled verification review, it should be updated, reviewed, signed and dated by the responsible person. Records should be kept in an organized manner with 'road maps' of where specific information can be found. For example, in the HACCP plan summary sheet, under the records column it may indicate that the CCP pasteurizer charts are kept in the supervisor's office; or in the written summary for the cleaning and sanitizing PP, it may indicate that checklists and chemical testing results are kept in a binder in the laboratory. Keeping as many records as practical in a centralized location facilitates the auditing process.

19.4   Other food safety systems

In addition to HACCP-based systems several other programs or procedures can be used to help ensure product safety as well as quality. The Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Foods (IOM/ NRC, 2003) lists other practices that can be used to identify, characterize and control potential risks associated with a specific food. These include using risk assessment techniques to identify and determine the level of a risk, establishing food safety objectives and performance criteria standards to define the acceptable level of a risk, and implementing statistical process control procedures to manage a risk. Additional programs that utilized and/or support HACCP systems and include

supply chain management components and recognition include the International Organization for Standardization (ISO, 2005) ISO 22000 standard for food safety management systems (FSMS), and benchmarked food safety schemes recognized under the Global Food Safety Initiative (GFSI) such as Safe Quality Food (SQF) and the British Retail Consortium (BRC). While beyond the scope of this chapter to discuss in detail, these tools may be used in conjunction with a HACCP based approach and will be described briefly.

Risk assessment is considered to be part of 'risk analysis', which also includes risk management and risk communication. Quantitative risk assessment 'is a scientific modeling process that addresses the magnitude of a risk and identifies factors that control it' (IOM/NRC, 2003). The process includes hazard identification, dose–response assessment, exposure assessment and risk characterization to determine the risk level of specific hazards associated with the food in question. An example is the quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods conducted by the FDA, the USDA and the CDC (FDA/USDA/CDC, 2003). Based on this model, unpasteurized milk was considered to be a high-risk food for listeriosis on a per serving basis (ranked fourth behind deli meats, uncooked frankfurters and pâté/meat spreads) and a moderate-risk food on a per annum basis (ranked seventh). Pasteurized fluid milk was considered a moderate risk on a per serving basis (ranked ninth) and a high risk on a per annum basis (ranked second behind deli meats).

Food safety objectives (FSOs) are established criteria for the maximum occurrence or concentration of a hazard in a food at the time of consumption such that illness or injury is unlikely (IOM/NRC, 2003). FSOs are generally established based on quantitative risk assessments, with emphasis on dose–response criteria, and take into account the process, as well as post-processing handling during transportation, at retail and by the consumer. Performance standards are applied at the processing operation and are set to ensure that the FSOs are met. The US has had a zero tolerance for *Listeria monocytogenes* in all ready-to-eat (RTE) foods; e.g., *L. monocytogenes* should be undetectable. For some RTE foods where *Listeria* growth is unlikely (e.g., frozen ice-cream), an acceptable level above zero may have limited risk, thus performance standards may be established that are greater than zero. In a draft guidance document on controlling *L. monocytogenes* in refrigerated or frozen RTE foods, FDA (2008a) recommends that *L. monocytogenes* be not present in ≥ 0.04 cfu per gram of food for RTE foods that support the growth of *L. monocytogenes*, e.g., not detectable in a 25 gram sample. This standard would apply to fluid milk products. For RTE foods that do not support the growth of *L. monocytogenes* (i.e., pH ≤ 4.4, water activity ≤ 0.92, or frozen), FDA recommends that foods entering commerce do not contain ≥ 100 cfu of *L. monocytogenes* per gram, thus suggesting a performance standard above 'zero tolerance'. This standard would not apply to fluid milk but may apply to frozen ice-cream.

Statistical process control (SPC) is used to ensure product safety and quality by controlling and monitoring the process over time to ensure that it is stable and

does not vary outside the acceptable limits. SPC operates on the assumptions that safety is built into the manufacturing process; the process can be monitored and the data can be analyzed with appropriate methods and statistical techniques; the process can be managed to ensure variation remains stable and predictable; and the process is able to consistently result in a product that meets performance standards (IOM/NRC, 2003). When applied correctly SPC can be used to monitor and predict the performance of the process and safety and quality of the product. With perhaps the exception of aseptic processes, SPC has not seen wide application in the fluid milk industry.

ISO Standard 22000:2005 (ISO, 2005) is a food safety management system that emphasizes communication along the food chain. It has a strong HACCP system component, is designed to ensure regulatory compliance and offers certification or registration that is recognized throughout the globe. ISO 22000 has a quality component and clearly defines prerequisite programs and their importance, beyond the basic HACCP system requirements. It defines 'operational PPs' as those used to control potential hazards that in their absence could result in economic or quality loss or low-risk health concerns (Surak and Wilson, 2007). Under the Global Food Safety Initiative (GFSI), four food safety benchmark schemes are currently recognized that allow supplier certification (CIES, 2008); these are Safe Quality Food (SQF), the British Retail Consortium, Dutch HACCP and the International Food Standard (IFS). For example, SQF is a program that is recognized in supply chain management with both HACCP based food safety and quality components and offers recognized food safety and quality management certification programs for primary producers (SQFI, 2005) and the entire food sector (SQFI, 2008). All those recognized under GFSI require specific food safety programs that include, but go beyond, a basic HACCP system approach.

19.5   Sources of further information and advice

19.5.1   Regulatory documents

Canadian Food Inspection Agency – Food Safety Enhancement Program:
   http://www.inspection.gc.ca/english/fssa/polstrat/haccp/haccpe.shtml
CODEX Alimentarius FAO/WHO Food Standards:
   http://www.codexalimentarius.net/web/index_en.jsp
FDA – HACCP:
   http://www.fda.gov/Food/FoodSafety/HazardAnalysisCriticalControlPoints HACCP/default.htm
FDA – Milk Safety References:
   http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/ MilkSafety/default.htm
New Zealand Food Safety Authority – Dairy:
   http://www.nzfsa.govt.nz/dairy/index.htm
US Code of Federal Regulations, Title 21:
   http://www.access.gpo.gov/cgi-bin/cfrassemble.cgi?title=200721

19.5.2   Trade Associations that provide information and training

Global Food Safety Initiative:
   http://www.ciesnet.com/2-wwedo/2.2-programmes/2.2.foodsafety.asp

Grocery Manufacturers' Association:
   http://www.gmabrands.com/about/index.cfm

International Dairy Foods Association:
   http://www.idfahaccp.org

19.6 References

ANON. (2008), 'National drug data base fiscal year 2007 annual report, October 1, 2006–
        September 30, 2007', GLH, Inc., Lighthouse Point, FL.

BERNARD D T, PARKINSON N G and CHEN Y (2006), 'Prerequisites to HACCP', in Scott V N
        and Stevenson K E (2006), *HACCP: A Systematic Approach to Food Safety*,
        Grocery Manufacturers Association, Washington, DC, pp. 5–12.

CAC (2003), 'Recommended international code of practice – General principles of food
        hygiene', CAC/RCP 1-1969, Rev. 4-2003, Rome: Food and Agriculture
        Organization of the United Nations, Codex Alimentarius Commission. Available
        at http://www.codexalimentarius.net/web/standard_list.do?lang=en, accessed 5
        May 2008.

CDC (2005), 'Human tuberculosis caused by *Mycobacterium bovis* – New York City,
        2001–2004', *MMWR*, 24 June, 54(24): 605–608.

CFIA (2007), 'Food Safety Enhancement Program manual', Canadian Food Inspection
        Agency. Available at http://www.inspection.gc.ca/english/fssa/polstrat/haccp/
        haccpe.shtml, accessed 17 May 2008.

CFIA (2008), 'Quality Management Program', Canadian Food Inspection Agency.
        Available at http://www.inspection.gc.ca/english/anima/fispoi/qmp/
        qmppgqe.shtml, accessed 2 April 2009.

CIES (2008), 'Global Food Safety Intiative', CIES – The Food Business Forum. Available
        at http://www.ciesnet.com/2-wwedo/2.2-programmes/2.2.foodsafety.gfsi.asp,
        accessed 2 April 2009.

EEC (1993), 'Council Directive 93/43/EEC on the hygiene of foodstuffs', European
        Economic Community.

FDA (2000), 'The interim report of the HACCP pilot evaluation team to the NCIMS-
        HACCP committee'. Available at http://www.cfsan.fda.gov/~comm/daieval.html,
        accessed 16 May 2008.

FDA (2005), 'Compliance policy guide section 555.425 – Foods – Adulteration involving
        hard or sharp foreign objects'. Available at http://www.fda.gov/ora/
        compliance_ref/cpg/cpgfod/cpg555-425.htm, accessed 16 May 2008.

FDA (2006a), 'Hazards and control guide for dairy foods HACCP, guidance for
        processors. Version 1.1. 2006'. Available at http://www.cfsan.fda.gov/~ear/
        daihaz.html, accessed 6 May 2008.

FDA (2006b), 'Milk plant equipment test report. Form FDA 2359b (10/06)', DHHS/FDA.
        Available at http://www.fda.gov/opacom/morechoices/fdaforms/FDA-2359b.doc,
        accessed 17 May 2008.

FDA (2007), 'Grade "A" Pasteurized Milk Ordinance (PMO), 2007 revision', Food and
        Drug Administration, US Department of Health and Human Services.

FDA (2008a), 'Control of *Listeria monocytogenes* in refrigerated or frozen ready-to-eat foods, draft guidance'. Available at http://www.cfsan.fda.gov/~dms/lmrtegui.html, accessed 9 June 2008.

FDA (2008b), 'Dairy grade ''A'' voluntary HACCP'. Available at http://www.cfsan.fda.gov/~comm/haccpdai.html, accessed 18 May 2008.

FDA/USDA/CDC (2003), 'Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods'. Available at http://www.cfsan.fda.gov/~dms/lmr2-toc.html, accessed 9 June 2008.

FDC (2009), 'HACCP & Regulatory', Food Development Center. Available at http://www.gov.mb.ca/agriculture/fdc/fdc04s06.html, accessed 2 April 2009.

GOMBAS K (2006), 'Prerequisite programs, foundation for HACCP', NCIMS HACCP industry orientation training presented in March 2006 in Syracuse, NY.

IDFA (2002), *IDFA's HACCP Plant Manual, 2002 edition*, International Dairy Foods Association, Washington, DC.

IOM/NRC (2003), 'Scientific criteria to ensure safe food', Institute of Medicine/National Research Council, The National Academies Press, Washington, DC.

ISO (2005), 'ISO Standard 22000:2005, food safety management systems – requirements for the organization in the food chain', International Organization for Standardization, Geneva.

JACOBUS C H, HOLICK M F, SHAO Q, CHEN T C, HOLM I A, KOLODNY J M, EL HAJJ FULEIHAN G and SEELY E W (1992), 'Hypervitaminosis D associated with drinking milk', *New England J. Med.*, 326: 1173–1177.

KIM S G, KIM E H, LAFFERTY C J and DUBOVI E (2005), '*Coxiella burnetii* in bulk tank milk samples', *United States Emerging Infectious Disease*, 11: 619–621.

MENDEZ MARTINEZ C, PAEZ JIMÉNEZ A, CORTÉS-BLANCO M, SALMORAL CHAMIZO E, MOHEDANO MOHEDANO E, PLATA C, VARO BAENA A and MARTINEZ NAVARRO J F (2002), 'Brucellosis outbreak due to unpasteurized raw goat cheese in Andalucia (Spain), January–March 2002', *Euro. Surveill.*, 8(7): pii=421. Available at http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=421, accessed on 16 May 2008.

NACMCF (1998), 'Hazard analysis and critical control point principles and application guidelines', National Advisory Committee on the Microbiological Criteria for Foods, *J. Food Prot.*, 61: 1246–1259.

NICHOLS J G (1992), 'Recommended levels of vitamins A & D in milk products', US Food and Drug Administration, Milk Safety Branch, M-I-92-13, 22 December 1992. Available at http://vm.cfsan.fda.gov/~ear/prime.html, accessed 16 May 2008.

NZFSA (1997), 'A guide to hazard analysis critical control point systems in the seafood industry'. Available at http://www.nzfsa.govt.nz/animalproducts/haccp/index.htm, accessed 17 May 2008.

NZFSA (2003a), 'Dairy and plants standard. D110.2 dairy HACCP plan. Circular number 76 dairy industry regulations 1990', New Zealand Food Safety Authority.

NZFSA (2003b), 'Operational guidelines dairy HACCP plants', New Zealand Food Safety Authority. Available at http://www.nzfsa.govt.nz/dairy/publications/guidelines/d110-guide.pdf, accessed 18 May 2008.

NZFSA (2004), 'A guide to HACCP systems in the meat industry', Available at http://www.nzfsa.govt.nz/animalproducts/haccp/index.htm, accessed 17 May 2008.

SCOTT V N and STEVENSON K E, EDITORS (2006), *HACCP: A Systematic Approach to Food Safety*, 4th edition, Grocery Manufacturers Association, Washington, DC.

SQFI (2005), 'SQF 1000 Code; a HACCP based supplier assurance code for primary producers, 5th edition', Safe Quality Food Institute/Food Marketing Institute. Available at http://www.sqfi.com/documentation/SQF1000_Code.pdf, accessed 9 June 2008.

SQFI (2008), 'SQF 2000 Code; a HACCP based supplier assurance code for the food industry, 6th edition', Safe Quality Food Institute/Food Marketing Institute. Available at http://www.sqfi.com/SQF_2000_Code_Edition6.pdf, accessed 2 April 2009.

SURAK J G and WILSON S (2007), *The Certified HACCP Auditor Handbook*, ASQ Quality Press, Milwaukee, WI.

US-CFR (2007a), '21 CFR Part 110 – Current good manufacturing practice in manufacturing, packing, or holding human food', Food and Drug Administration, Department of Health and Human Services. Available at http://www.access.gpo.gov/nara/cfr/waisidx_07/21cfr110_07.html, accessed 18 May 2008.

US-CFR (2007b), '21 CFR Part 120 – Hazard analysis and critical control point (HACCP) systems', Food and Drug Administration, Department of Health and Human Services. Available at http://www.access.gpo.gov/nara/cfr/waisidx_07/21cfr120_07.html, accessed 18 May 2008.

US-CFR (2007c), '21 CFR Part 123 – Fish and fishery products', Food and Drug Administration, Department of Health and Human Services. Available at http://www.access.gpo.gov/nara/cfr/waisidx_07/21cfr123_07.html, accessed 18 May 2008.

US-CFR (2008), '9 CFR Part 417 – Hazard analysis and critical control point (HACCP) systems', Food and Drug Administration, Department of Health and Human Services. Available at http://www.access.gpo.gov/nara/cfr/waisidx_08/9cfr417_08.html, accessed 18 May 2008.

Index