Meera Kaur

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ISBN: 978-0-387-79377-1 e-ISBN: 978-0-387-79378-8
DOI: 10.1007/978-0-387-79378-8

Library of Congress Control Number: 2008935081

Printed on acid-free paper

springer.com

To my parents
Late Prabin C. Chakravarty
and
Ms. Sarala Chakravarty

Preface

In the last couple of decades, a revolution has occurred in the area of medical nutrition therapy; particularly, the role of basic and conditionally essential nutrients in the management and treatment of various diseases has received significant attention. As a result, several research articles and books have been published in the area of medical nutrition therapy. Simultaneously, medical foods (enteral foods) have overwhelmingly flooded the global market. In such a situation, a question arises: why to write another book on *Medical Food from Natural Sources?* What is the contribution of this book to medical nutrition therapy?

First, what is new in this book is the development of the medical (enteral) foods from *natural sources*. Because of the prevalent *drug-like approach* to the formulation of medical foods, most of the enteral foods marketed currently are a blend of defined or chemically defined food ingredients. These ingredients lack the natural stimulants and protectants present in normal foods. Further, they are unpalatable and often artificially flavored and excessively sweetened to mask the chemical taste of the ingredients. Furthermore, the defined ingredients-based enteral foods are too expensive for low-income-group patients.

To overcome the aforementioned drawbacks associated with defined ingredients-based enteral foods, this book describes the development of cost-effective enteral foods from natural sources such as barley, rice, mung bean, eggs, milk, etc. True, the preparation of nutrient-dense liquid foods from the natural food ingredients that can flow easily through feeding tube can be a difficult task for the scientists. To overcome this difficulty, this book suggests the use of malted cereals and grain legumes as bases for the preparation of enteral foods. This is where this book offers a unique contribution to the *food-like approach* to develop *medical foods*, which is an alternative to conventional *drug-like* approach.

Second, to the best of my knowledge, none of the published books in medical nutrition therapy offer information relating to the developmental aspects of enteral foods. These aspects play a critical role in overall acceptability, tolerance, and effectiveness of enteral nutrition support. Therefore, this book fills this prominent gap in the literature by addressing in detail the developmental aspects of enteral foods.

In sum, this book encourages the readers to combine the principles of food science with food technology to develop a cost-effective enteral nutrition support to the

patients. I sincerely hope that this research-based information will spark new ideas in the readers interested in the development of *medical food from natural sources*. Your comments are most welcome at dr_mkaur@hotmail.com.

Winnipeg, Canada Meera Kaur

Acknowledgments

I sincerely thank the following professors, scientists and professionals for their guidance and suggestions in the preparation of this book: N.G Malleshi, CFTRI, India; Satyendra Singh, Athar Ata, University of Winnipeg, Canada; G.L. Kaul, Assam Agricultural University, India; Donald Duerksen, St. Boniface General Hospital, Canada; Analyn Baker, Stefania Zimarino, Seven Oaks General Hospitals, Canada; Evelyn Boyko, Health Sciences Centre, Canada; Gayotri Goswami, Albert Einstein College of Medicine, USA; Ashok Chacko, George M. Chandy, CMC Hospital, India; M. Markandayalu, Osmania General Hospital, India; V. Lakshmi, Nizam's Institute of Medical Sciences, India; Jose Mathew, C.S.I. Holdsworth Memorial Hospital, India; G. Ramanathan, Kovai Medical Centre and hospital, India; and, Varsha, ISPEN, India. It was my good fortune to work with the editors: Susan Safren, David Parsons, Jeffrey Taub, Springer, USA. My thanks are due to them for their all support. Finally, It was nice to work with Mr. S. Prasad, SPi Technologies India Private Ltd., India—many thanks to him.

Note on the Author

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Contents

List of Figures

List of Tables

List of Abbreviations

AAA	Aromatic Amino Acids
AACC	American Association for Clinical Chemistry
AAS	Amino Acid Score
AF	Aluminium Foil Laminate
ARF	Amylase Rich Food
AOAC	Association of Official Agricultural Chemists
BC	Before Christ
BCA	Bacillus Cereus Selective Agar
BCAA	Branched Chain Amino Acids
BMR	Basal Metabolic Rate
BPA	Bisphenol A [2, 2-bis(4-hydroxyphenyl)] Propane
BSA	Bovine Serum Albumin
BSS	British Standard Specification
BUN	Blood Urea Nitrogen
BV	Biological Value
CMI	Cell Mediated Immunity
COPD	Chronic Obstructive Pulmonary Diseases
cP	Centipoises Unit
Cmm	Cubic Millimetre
DEF-BP	Disease-specific Enteral Food for Burn Patient
DHA	Docosahexaenoic acid
DMSO	Dimethylsulphoxide
DNS	Dinitrosalicylic acid
DS	Dextrose Starch
Dwb	Dry Weight Basis
EAA	Essential Amino Acids
EDTA	Ethylene-Diamine-Tetra-Acetic acid
EFA	Essential Fatty Acids
EMB	Eosin Methylene Blue Agar
EN	Enteral Nutrition
EPA	Eicosapentaenoic Acid

FALCA	Food Allergen Labeling and Consumer Protection Act
FAO	Food and Agriculture Organization
FFA	Free Fatty Acid
FFDCA	Federal Food, Drug, and Cosmetic Act
g	gram
GEF-LC	General-Category Low-Cost enteral food
GEF-RT	General-Category Ready-To-eat enteral food
GIT	Gastro-Intestinal Tract
GLC	Gas Liquid Chromatography
GPC	Gel Permeation Chromatography
GRAS	Generally Recognized as Safe
h	Hour
Hb	Hemoglobin
HCV	Hepatitis C Virus
HI	Humoral Immunity
HTST	High Temperature Short-Time Treatment
IBD	Irritable Bowel Disease
IgA	Immunoglobulin A
IGFBP	Insulin-like Growth Factor Binding Protein
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ISI	Indian Standard Institution
IU	International Unit
IV	Intravenous
kcal	Kilocalorie(s)
Kd	kilodalton.
KF	Kang-Fung
kg	Kilogram(s)
LCEF	Low Cost General-Category Enteral Food
LTST	Low Temperature and Short Time
LAB	Lactic Acid Bacteria
LMIT	Lymphocyte Migration Inhibition Test
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean Corpuscular Volume
MCT	Medium-Chain Triglycride
ME	Mercaptoethanol
MEq:	Millieqivalent
MIT	Migration Inhibition Test
MoDC	Monocyte-derived Dendritic Cells (MoDC)
μg	Microgram
mg.	Milligram

ml.	Milliliter
MP	Metallised Polyester
MRS	Mann-Rogosa-Sharpe
MTH	Methylhistidine
MUG	4-Methylumbelliferyl-Beta-D-Glucuronide
NASA	National Aeronautics and Space Administration
NG	Nasogastric Feeding
NPR	Net Protein Ratio
NPU	Net Protein Utilization
OTR	Oxygen Transmission Rate
PCV	Packed cell volume
PDA	Potato Dextrose Agar
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PEF	Proprietary Enteral Food
PEG	Percutaneous Endoscopic Gastrostomy
PEJ	Percutaneous Endoscopic Jejunostomy
PER	Protein Efficiency Ratio
PHA	Phytohemagglutinin
PN	Parenteral Nutrition
PUFA	Poly Unsaturated Fatty Acids
RBC	Red Blood Cells
RSL	Renal Solute Load
SDS	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SEM	Scanning Electron Microscopic
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SSA	Salmonella Shigella Agar
TBSA	Total Burn Surface Area
TD	True Digestibility
TLC	Total lymphocyte count
TPC	Total Plate Count
TPN	Total Parenteral Nutrition
WBC	White Blood Cells
WSI	Water Solubility Index
WVTR	Water Vapor Transmission Rates

Chapter 1
Introduction

Introduction

Proper nutritional support to patients improves their responsiveness to the medical treatment, reduces morbidity and mortality, and cuts the hospitalization costs significantly. There are two kinds of nutrition support – parenteral and enteral. Parenteral nutrition support is effective and lifesaving, but its prolonged use is not desirable because it fails to preserve gut functions and integrity. However, when the gastrointestinal tract is not functional, parenteral nutrition support (i.e., feeding sterile nutrients via vein, thereby bypassing the gut) still remains as an alternative method of nutritional support. By contrast, enteral nutrition support (feeding food orally or via a tube to the gut) is an effective method to provide nutritional support to patients who have a functional or partially functional gastrointestinal tract but are unable to nourish themselves effectively. Enteral nutrition support has gained significant importance over parenteral nutrition, because it preserves intestinal structure and functions. Further, it is cost effective, easy to administer and manage. This book is designed to contribute to effective nutrition support to patients by developing the enteral food from natural sources.

Enteral foods are a class of liquid medical foods that are engineered to provide complete or partial enteral nutritional support to the patients. These foods are delivered either orally or through a tube directly to the stomach, duodenum, or jejunum, depending upon the medical condition of the patient. Although several enteral foods are available in the market, most of them are based on either defined or chemically defined ingredients wherein maltodextrin, glucose, or fructose serve as sources of carbohydrates; sodium or calcium caseinate, amino acids or protein hydrolysates as sources of protein; and, soy oil, corn oil, medium chain triglycerides, or structured lipids as sources of lipid. These defined or chemically defined ingredients are expensive and lack the natural stimulants and protectants that are present in the natural foods. Further, to mask the off flavor of defined ingredients, these foods often need to be artificially flavored. Furthermore, some of the defined ingredients do exert high osmotic load causing diarrhea or dehydration.

To overcome the aforementioned issues, this book explains the process for the development of a range of cost-effective enteral foods, akin to normal wholesome

M. Kaur, *Medical Foods from Natural Sources,*
DOI: 10.1007/978-0-387-79378-8_1, © Springer Science+Business Media, LLC 2009

diet, from *natural food* ingredients. Controlled clinical trials carried out on burn patients and case studies conducted on patients suffering from various diseases confirmed better acceptability and tolerance of the natural ingredients-based enteral foods than defined ingredients-based formulae. In addition to providing optimal nutritional support, natural ingredients based-enteral foods were cost effective compared with defined ingredients-based enteral foods. Clearly, it is worthwhile to disseminate the *new* knowledge and *in*expensive technology for the development of enteral food from natural sources to the intellectual community. Certainly, application of this technology will contribute significantly to the nutritional management of patients around the world while reducing the total costs of patient care system.

Given the importance of enteral nutrition in medical sciences, several books have been published relating to enteral nutrition support. But most of these published books focus *only* on the issues such as rationale for enteral nutrition support, specific nutrient requirements for various disease conditions, practical approaches to the delivery, and monitoring and prevention of complications while providing enteral nutrition support. None of them offers information relating to the developmental aspects of enteral foods (e.g., processing technology, types of ingredients, physicochemical and nutritional characteristics, and shelf-life evaluations, among others). These aspects are critical because they affect the overall acceptability, tolerance, and effectiveness of enteral nutrition support. Therefore, a need was felt to fill the gaps in the literature by addressing the developmental aspects of enteral foods in detail, such as processing technologies, physicochemical quality evaluation of processed ingredients and the formulations based on them, shelf-life evaluation, and clinical outcome studies of natural ingredients-based enteral foods.

Hence, the purpose of writing this book is to communicate the novel technology for developing enteral foods from natural sources, discuss the results of the clinical trials conducted in various categories of patients, and evaluate the efficacy of the natural ingredients-based enteral foods. This research-based book addresses the aforementioned issues and serves the need of patients in both developed and developing countries through this inexpensive yet effective enteral nutrition support.

Ideally, the book has been written for postgraduate and research students. However, some aspects of this book will serve the upper level undergraduate students. Also, this book is intended for researchers, scientists, medical fraternity, or anybody who is interested in advancing knowledge in the area of enteral foods and enteral nutrition support. Because this book covers two different yet related areas – Food science and Technology, and Clinical Nutrition – it caters two sets of audiences.

From *Food Science and Technology* viewpoint, this book has been written at postgraduate level making it suitable for students, scientists, or researchers in the area of food science, grain science and technology, milling technology, sensory sciences, and dairy technology, among others. This book explains various food processing technologies such as how to a prepare enzyme-rich, fiber-regulated flour from cereals and grain legumes suitable for enteral foods; resist the coagulation characteristics of heat-treated eggs making them suitable for enteral-tube feeding; incorporate live probiotics and active bacterial cultures in enteral and other

specialty foods; formulate enteral food from natural ingredients; and, evaluate the physicochemical and nutritional qualities of enteral foods. In addition, description of detailed methodologies to evaluate the packaging and shelf-life of enteral foods makes the book ideal for students at postgraduate and research levels. Furthermore, the in-depth explanation of the textural, physicochemical, and molecular changes occurring during the processing of food grains positions the book as a valuable reference book for reference librarians.

From *Clinical Nutrition* viewpoint, the book serves as an excellent book for classroom teaching and applied research at undergraduate and postgraduate levels. Some of the topics in this book also relate to medical nutrition therapy such as history of enteral nutrition, need for specialized nutrition support, changes in the gut physiology during starvation and illness, and enteral vs. parenteral nutrition support, among others. Further, the detailed explanation of the study design to conduct controlled clinical trials and case studies, and practical approaches to evaluate the acceptability and tolerance of natural ingredients-based enteral foods should serve as a model to develop research project at both undergraduate and postgraduate levels. In sum, this book provides the reader with in-depth information about the stages of development and utilization of the enteral food from natural sources. A brief description of the subsequent chapters of this book is presented in the following paragraphs.

Chapter 2 introduces the subject of enteral nutrition, enumerates the history of enteral nutrition, states the merits of enteral nutrition over parenteral nutrition, and explains the gut physiology during enteral and parenteral nutrition support. Next, this chapter discusses some of the food processing technologies such as malting, popping, toasting, and spray drying that are applicable to enteral food formulation from natural food ingredients. Further, the chapter explains about removal or reduction of antinutritional factors that are naturally present in some foods by employing suitable food processing technologies. Furthermore, the textural, nutritional, and physiochemical qualities of processed ingredients and the malt-based specialty foods are discussed in detail. Specifically, the chapter answers the question: What is the rationale for the development of enteral foods from natural sources?

Chapter 3 explains the methodology for the preparation of rice and barley malt, and milling of rice and barley malt to prepare enzyme-rich and fiber-regulated flour. Cereal malt is a primary product that requires further processing for food uses and value addition. Malting of barley has been studied, and various unit operations involved in industrial malting have been standardized. But, information on malting of rice is scanty, albeit limited literature on rice malt reveals that the germinated rice possesses both liquefying and saccharifying enzymes, which could be a good cereal base for specialty foods. This chapter illustrates the novel technology developed for rice malting, and milling of malted rice to prepare enzyme-rich, fiber-regulated flour. Quality characteristics of the malted flours such as viscosity, amylase activity, changes in carbohydrate and protein molecules, and dietary fiber contents are discussed. Further, the processing technologies of other natural ingredients suitable for the enteral food formulations (e.g., amaranth seed, mung bean,

soybean, milk, egg, and gum acacia) are presented. In addition, the desirable changes occurring during processing of these food ingredients are discussed.

In short, this chapter answers these questions: (1) Can the textural, physicochemical, and nutritional characteristics of cereals and grain legumes be modified by in-vivo biotransformation technology such as controlled malting? (2) Is it possible to prepare enzyme-rich, fiber-regulated flour from cereal and grain legumes, suitable for preparing medical foods and other specialty foods?

Chapter 4 describes the various unit operations involved in the development of general-category and disease-specific enteral foods from natural sources. In brief, these operations include formulation, blending of raw materials, mashing, cooking, homogenization, and spray drying of the foods. Processing technologies of the three natural ingredients-based enteral foods are described in this chapter. They are (1) a low-cost general-category enteral food, (2) a ready-to-eat general-category enteral food, and (3) a disease-specific enteral food for burn patients and patients at hypercatabolic state. Also, this chapter elaborates the physicochemical parameters, nutritional qualities, microbiological safety, and pesticide-residuals safety of these three natural ingredients-based enteral foods.

In nutshell, this chapter attempts to answer the following queries: (1) Can the processed cereals and legumes be gainfully utilized for the development of nutritionally balanced, energy-dense, and cost-effective enteral foods? (2) Is it possible to alter the coagulation characteristics of heat-treated egg protein to prepare ready-to-eat, egg-based enteral foods suitable for tube feeding? (3) Is it possible to fortify enteral food with live probiotics? (4) Are the nutrient compositions and the physicochemical characteristics of the natural ingredients-based enteral foods comparable with the enteral foods based on defined ingredients? And, (5) What are the advantages of natural ingredients-based enteral foods over the defined ingredients-based enteral foods?

Chapter 5 explains the methodology for the evaluation of protein quality of the enteral foods in animal models, highlights the growth promotion and nitrogen balance in animals, and discusses the histology of the liver tissues of animals fed with enteral food formulations. The protein quality of the enteral formulations was evaluated in terms of protein efficiency ratio (PER), net protein ratio (NPR), biological value (BV), true digestibility (TD), amino acid score (AAS), amino acid rating (AAR), and protein digestibility corrected amino acid score (PDCAAS). The results are discussed in detail in this chapter. This chapter answers the following question: (1) Is the protein quality of the formulated enteral foods comparable with that of defined ingredients-based enteral foods?

Chapter 6 enumerates the sorption behavior and shelf-life of the enteral foods, and explains the methodologies used to evaluate the shelf-life of the enteral foods. The effects of different variables such as packaging materials, packaging conditions, temperature, and humidity on the shelf-life of enteral foods are discussed with statistical analyses. Changes observed in sensory attributes, physicochemical parameters, and microbiological safety of the enteral food during storage are also presented. This chapter seeks to answer the following questions: (1) What are the best packaging materials for storage of

the enteral foods? (2) What is the optimum storage condition for such food? (3) Do the specific nutrients or functional components (e.g., active bacterial cultures added to the enteral foods) remain viable during storage?

Chapter 7 presents the methodology followed and results obtained from the controlled clinical trials conducted on selected burn patients to evaluate the efficacy of the disease-specific enteral food developed for burn patients. The various steps involved in conducting the prospective clinical trials (e.g., protocol development, ethical clearance, and consent from the patients, among others) are presented in detail in this chapter. The physiological and biochemical insults that occur during burn injury are enumerated with reference to the recent literature. The role of specific nutrients in the nutritional support to thermally injured patients is discussed. Further, this chapter focuses on the changes in the immune system during severe burn injury, and the role of various immune-enhancing nutrients in boosting the immune status of burn patients. In short, this chapter answers the following questions: (1) What are the physiological consequences of burn injury in relation to the total body surface area burn? (2) Are glutamine fish oil and probiotics useful in nutritional support to burn patients? (3) Is natural ingredients-based enteral food effective in providing the optimal nutritional support to burn patients?

Chapter 8 includes the methodologies and results of case studies conducted on various patients to evaluate the acceptability, tolerance, and overall efficacy of the two general-category enteral foods. In each case study, the following aspects are discussed: (1) What is the rationale for providing enteral nutrition support relevant to the specific patient? (2) Do natural ingredients-based enteral foods provide optimal nutritional support to the patients? (3) Is natural ingredients-based enteral food accepted and tolerated better than defined ingredients-based enteral food? (4) Is there any drawback of the low-cost general-category enteral food in providing nutrition support to patients at home or hospital?

Chapter 9 summarizes this book and provides implications of this study for health professionals along with directions for future research.

Chapter 2
Nutrition Support During Illness

Introduction

The knowledge of human nutrition does not only prevent deficiency diseases but also helps manage many diseases. One of the most important advances in modern medicine is better understanding of the role of basic and conditionally essential nutrients including fluid and electrolytes in the nutritional management of patients. Proper nutritional support to patients at large and hospitalized ones in particular plays an important role in effective medical treatment, speedy recovery, reducing the hospital stay and the medical expenses, besides decreasing the mortality and morbidity rate. Hence, a number of medical foods have been developed and marketed throughout the world. This chapter introduces the subject of medical and enteral nutrition, enumerates the history of enteral nutrition, states the merits of enteral nutrition over parenteral nutrition, and explains the gut physiology during enteral and parenteral nutrition support. Further, this chapter discusses some of the food processing technologies such as malting, popping, toasting, and spray drying that are applicable to enteral food formulation from natural food ingredients.

What Is Medical Food?

Medical Foods

Medical Foods are specially processed or formulated foods that are used for the dietary management of patients. They are intended for the exclusive or partial feeding of patients with limited or impaired capacity to ingest, digest, absorb, or metabolize ordinary foodstuffs or certain nutrients contained therein, or who have other special medically determined nutrient requirements, whose dietary management cannot be achieved only by modification of the normal diet, or by other foods for special dietary uses, or by a combination of two (Codex Alimentarious, 1994).

The US Food and Drug Administration (FDA, 2007) has defined medical food as "a food which is formulated to be consumed or administered enterally under the

M. Kaur, *Medical Foods from Natural Sources,*
DOI: 10.1007/978-0-387-79378-8_2, © Springer Science+Business Media, LLC 2009

supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation." The agency advises the consideration of the statutory definition of medical foods to narrowly constrain the types of products that fit within this category of food. The following paragraphs summarize the FDA's current guidelines on medical foods and should be viewed only as recommendations unless specific regulatory or statutory requirements are cited.

Medical foods are distinguished from the broader category of foods for special dietary uses and intended to meet distinctive nutritional requirements of a disease or condition, used under medical supervision. The term *medical foods* does not pertain to all foods fed to sick patients. Medical foods are foods that are specially formulated and processed (as opposed to a naturally occurring foodstuff used in a natural state) for the patient who is seriously ill or who requires the product as a major treatment modality. In general, to be considered as a medical food, a product must satisfy certain criteria such as the product must be a food for oral or tube feeding; labeled for the dietary management of a specific medical disorder, disease, or condition for which there are distinctive nutritional requirements; and, intended to be used under medical supervision.

Medical foods are exempted from the labeling requirements for health claims and nutrient content claims under the Nutrition Labeling and Education Act. However, being a food, its label must contain a statement of identity (name of the product); an accurate statement of the net quantity of contents; the name and place of the manufacturer, packer, or distributor; and a complete list of ingredients listed by their usual name in descending order of predominance. In addition, all words, statements, and other information required by or under authority of the Federal Food, Drug, and Cosmetic Act (FFDCA) must appear with prominence and conspi-cuousness and be in English except that for medical foods distributed solely in the Commonwealth of Puerto Rico or in a Territory where the predominant language is other than English. If a label bears any representation in a foreign language, then all mandatory label information must be repeated in each foreign language used on the label. Also, medical foods must be labeled in conformance with the principal display panel requirements, the information panel requirements, and the misbranding of food requirements.

In addition, Medical foods must comply with all applicable requirements for the manufacture of foods, including the Current Good Manufacturing Practices regulations, Registration of Food Facilities requirement, if applicable, the Low Acid Canned Food regulations, and Emergency Permit Control regulations. Ingredients used in medical foods must be approved food additives or a food additive that is the subject of an exemption for investigational use, if the ingredients are not generally recognized as safe (GRAS). Furthermore, Food Allergen Labeling and Consumer Protection Act (FALCA, 2004) requires that medical foods labeled after January 1, 2006 include on the label the food sources of the major allergens such as milk, eggs, fish bass, flounder, cod, crab, lobster, shrimp, almonds, pecans, walnuts, wheat, peanuts, and soybeans, among others.

Medical foods do not have to undergo premarket review or approval by FDA, and individual medical food products do not have to be registered with FDA. However,

FDA's compliance program for medical foods enables FDA inspectors to (1) obtain information regarding the manufacturing/control processes and quality assurance programs employed by domestic manufacturers of medical foods through establishment inspections, (2) collect domestic and import surveillance samples of medical foods for nutrient and microbiological analyses, and (3) take action when significant violations of the Federal Food, Drug and Cosmetic Act (or related regulations) are found.

Enteral Foods

Enteral foods are a class of liquid medical foods that are specially formulated to provide complete or partial nutritional support to the patients whose gut functions are fully or partially normal with accessible gastrointestinal tract (GIT), but are unable to ingest adequate quantity of food and meet their requirements orally. These foods are delivered either orally or through a feeding tube directly to the stomach, duodenum, or jejunum.

Routes of Delivering Enteral Nutrition Support

Routes of delivering enteral nutrition support depend upon the specific need of the patient, functioning and capacity of the gastrointestinal (GI) tract, underlying disease states, patient's tolerance, and the duration of the feeding requirement. Factors influencing selection of a specific route, their advantages, and disadvantage are briefly presented in the subsequent paragraphs.

Nasogastric Feeding

In case of nasogastric (NG) feeding, the feeding tube is inserted through the nose down the throat and esophagus into the stomach. The patient should have a functional stomach, an intact gag reflex with no esophageal reflux, and normal gastric emptying. The advantages of NG feeding are easy insertion of the feeding tube and large reservoir capacity in the stomach uninvolved with the primary disease. The major disadvantages are risk of pulmonary aspiration, and usually patients are self-conscious due to appearance of the NG feeding tube.

Nasoduodenal Feeding

Nasoduodenal is the route where the feeding tube through the nose extends further up to the duodenum (first part of the intestine). Nasoduodenal feeding is usually recommended to the patients with gastroparesis or impaired gastric emptying and

esophageal reflux. The major advantage of nasoduodenal feeding over NG feeding is the reduced risk of aspiration. Disadvantages of this type of feeding may include endoscopic placement of nasoduodenal tube, chance of tube displacement, and GI intolerance to goal tube-feed (TF) infusion rate. Patients are usually self-conscious due to appearance of the nasoduodenal feeding tube.

Nasojejunal Feeding

In nasojejunal feeding, the feeding tube is placed through the nose down to the jejunum (second part of the small intestine). Patients having gastric dysfunction due to trauma or surgery, impaired gastric emptying, and esophageal reflux may be fed through this route. Advantages of this route are that feeding can be initiated immediately after injury. Moreover, it has reduced aspiration risk compared with NG feeding. Disadvantages are similar to that of nasoduodenal feeding.

The earlier three routes are generally used to provide short-term nutritional support to patients. For long-term nutrition support *gastrostomy* and *jejunostomy* are the preferred route for enteral nutrition support.

Gastrostomy Feeding

In gastrostomy, the tube is inserted surgically through the abdominal wall into the stomach. Conditions that warrant gastrostomy feeding are patients with swallowing dysfunction and subsequent impairment of ability to consume an oral diet, and unavailability of the nasoenteric route. Usually, these patients have normal gastric emptying with a stomach uninvolved with the primary disease, intact gag reflex, and no esophageal reflux. One of the advantages of this route is that it can be performed adjunctly with GI surgery. Further, it has decreased risk of tube occlusion due to a large-bore tube, and large reservoir capacity in stomach. Disadvantages are potential risk of aspiration, chances of infection at the stoma site, risk of skin excoriation from leakage of digestive secretions at the stoma site, and potential risk of fistula after removal of the tube.

When the gastrostomy is made percutaneously (through the skin) using an endoscope (a flexible, lighted instrument) to determine where to place the feeding tube in the stomach and secure it in place, it is known as percutaneous endoscopic gastrostomy (PEG). One of the advantages of PEG is that the feeding tube can be placed without having to perform an open operation on the abdomen (laparotomy). In addition, PEG takes less time, carries less risk, and costs less than a classic surgical gastrostomy, which requires opening of the abdomen. Therefore, when feasible, PEG is preferable to a classic gastrostomy. The possible complications of PEG include wound infection (as in any kind of surgery) and dislodging or malfunction of the tube.

Jejunostomy Feeding

In jejunostomy, the tube is surgically inserted through the abdominal wall and extends up to the jejunum. Patients with high risk of aspiration, esophageal reflux, impaired gastric emptying, or gastric dysfunction due to trauma or surgery are usually fed with jejunostomy. This route is used when the upper GI tract must be bypassed completely. A jejunostomy tube can be used as soon as 12 h after surgery. This type of tube is usually used for people who have stomach ulcers. Advantages are similar to that of gastrostomy except that jejunostomy has small sized bore tube. Disadvantages are also similar to that of gastrostomy.

Percutaneous endoscopic jejunostomy (PEJ) is a surgical procedure for placing a feeding tube into the jejunum without having to perform an operation opening the abdomen. The advantages and disadvantages of PEJ are similar to that of PEG.

Methods of Enteral Tube Feeding

Enteral tube feedings are administered either as a bolus, continuous or intermittent feeding (Parsa et al., 1989).

Bolus Tube Feeding

In bolus feeding, usually around 200–250 mL of food is given at a time through a large syringe. It has advantages of requiring less amount of time, few equipment, and reduced risk of contamination. However, there are more chances of aspiration after a bolus feed. Patients with short bowel or malabsorption syndrome, who run the risk of physiologic intolerance to the bolus of carbohydrate, protein, or fat, do not tolerate this type of feeding.

Continuous Tube Feeding

In Continuous tube feeding, a predetermined quantity of food per hour is provided continuously. This method is used to prevent GI intolerance and to minimize the risk of aspiration. This type of feeding has the advantages of leaving the smallest residual volume and has the least potential for aspiration, bloating, and diarrhea. However, it requires 12–72 h to reach the tube feeding goal rate. Moreover, it needs close monitoring and has the danger of causing bacterial overgrowth.

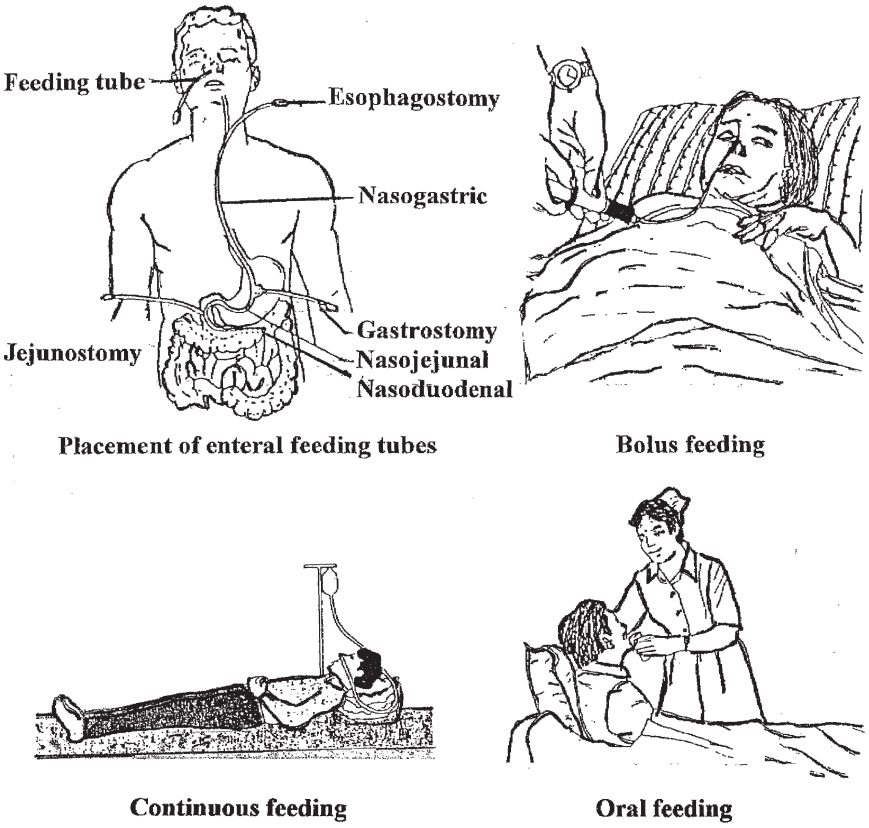


Fig. 2.1 Route and methods of enteral feeding

Intermittent Tube Feeding

Intermittent tube feeding is provided intermittently in between the oral intake. This type of feed is usually well tolerated and may be used in stable patients who have adequate absorptive capacity to tolerate bolus feeding. An enteral infusion device (feeding pump) may enhance the safety and accuracy of continuous and intermittent enteral feeding. Figure 2.1 shows the routes and methods of enteral feeding.

Patients Requiring Enteral Nutrition Support

Approximately 25–50% of the hospitalized patients is malnourished and requires additional nutrition support (Roubenoff et al., 1987). Enteral foods are tailored to provide adequate protein, calorie, protective nutrients, and other conditionally

essential nutrients to the patients of general medicine and surgery such as *patients at ICU* (Doyle and Kennedy, 1994), *patients with malignancy* (Raykher et al., 2007), *comatose* (Wong et al., 1997; Bochicchio et al., 2006), *sepsis* (Forse and Chavali, 1995), *dysphasia* (Kaur, 2007), *anorexia, severe diarrhea* (Mark and Stalker, 1993), *malabsorption, children with congenital heart diseases* (Schwarz et al., 1990), and geriatric patients. Extended or specific nutritional support to patients suffering from burn injury (Ahuja and Goel, 1993; Chakravarty et al., 2002), renal and liver dysfunction, pulmonary diseases and many other clinical disorders (Randall, 1990), including AIDS patients (Cope et al., 1995; Ockenga et al., 2006) is also provided through enteral nutrition.

In the developed countries, enteral nutrition support has become part and parcel of medical care. According to Schmidl and Labuza (1992), more than 5-million patients including many of the normal geriatrics are put on enteral nutrition annually in USA, and out of that, 0.7 million are on exclusive tube feeding. The estimated market value of these enteral foods exceeds $1 billion annually (FASEB, 1991; Campbell, 2006). As per Silk et al. (1992), the major share of the enteral foods produced in UK is utilized by patients at ICU (37%), followed by patients of general medicine (20%), postoperative patients (20%), geriatrics (10%), burns (7%), and preoperative patients (6%).

During the last decade, enteral nutrition support has received attention in developing countries as well. However, statistics regarding the percent of various patients requiring enteral nutrition support in these countries do not seem to be yet reported.

Contraindication to Enteral Nutrition Support

As per the guidelines of American Society for Parenteral and Entral Nutrition (ASPEN, 2002), enteral nutrition support is contraindicated in certain conditions such as gastrointestinal obstruction, prolonged ileus, severe diarrhea or vomiting, enterocutaneous fistula, incomplete resuscitation, inadequate bowel surface area, feeding intolerance, and unattainable enteral access.

Parenteral Nutrition

Webster's New World Medical Dictionary defines total parenteral nutrition (TPN) as intravenous feeding that provides patients with all of the fluid and the essential nutrients they need when they are unable to feed themselves by mouth. Generally, during critical illness and also in many physiological situations, the patients are provided with parenteral nutrition (PN), wherein fully predigested liquid sterile nutrients of varying strength are administered directly into the circulation (intravenous). The absolute indication of parenteral nutrition support is when the patient has a nonfunctioning GI tract. Although, TPN is very effective and could be lifesaving at times, its prolonged use is not desirable because it fails to preserve gut functions

and integrity, causes villus atrophy (Fig. 2.2), reduces the activity of disaccharidase, and decreases the cellularity that may cause curling ulcer (Rombeau et al., 1997). It may also result in the overgrowth of pathogenic microflora in the intestine and the disruption of gut barrier functions, thereby causing bacterial translocation leading to severe sepsis. Hence, greater emphasis is paid to enteral nutrition because it preserves intestinal structure and functions and is easy to administer and manage. Moreover, enteral nutritional support is cost effective. The comparative merits of enteral nutritional support over parenteral nutrition are presented in Table 2.1.

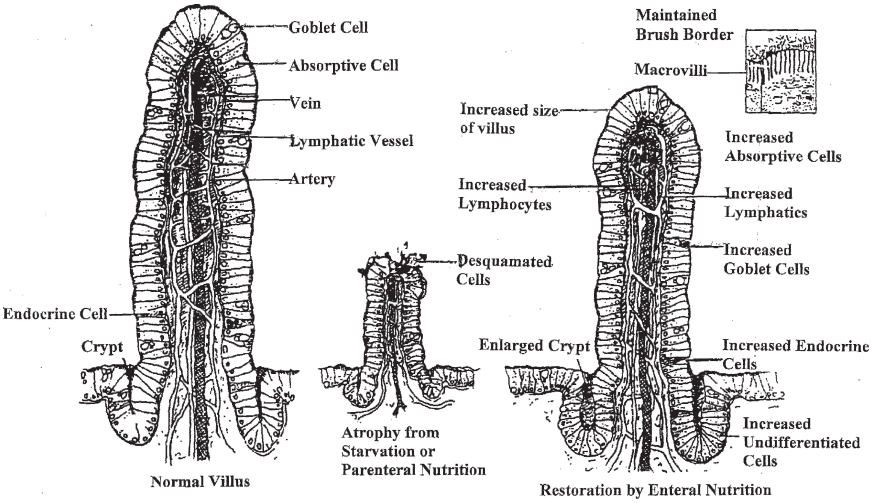


Fig. 2.2 Effect of parenteral and enteral nutrition support on intestinal epithelium and villus structure

Table 2.1 Merits of enteral nutrition support over parenteral nutrition support

Enteral nutrition	Parenteral nutrition
Easy to administer, more physiological, and adaptive to gut functions	Difficult to administer and more mechanical
Normal status of the intestinal villi is maintained, and the production of undifferentiated cells in the crypts is increased	Leads to villus atrophy, decreases cellularity and a reduction in the intestinal disaccha-ridase activity
Preserves and maintains intestinal structure and functions	Fails to preserve intestinal structure and functions
Septic complications are rare	Incidences of serious infections, mainly catheter-related sepsis occur
Preserves mucosal barrier and prevents bacterial translocation	Causes physical disruption of the mucosal barrier and enhances translocation of the bacteria and endotoxin
Maintains normal liver functions and prevents gall bladder motility and biliary sludge or lithiasis	May effect liver functions and cause metabolic complications
Enhances immunocompetence	Acts as a form of iatrogenic immunosupperssion
Cost effective	Quite expensive

History of Enteral Nutrition

The importance of enteral feeding for preserving good health was recognized even many years BC and the Egyptian and Greek physicians used grain broth, milk, chicken broth, and wine for rectal feeding the needy. Later in the fifteenth century, enteral feeding through nostril using hollow silver tube inserted up to esophagus was followed. Developments in the enteral nutrition continued over the years and in early 1980s, US physicians used flexible rubber tube for delivering the food into the gut. Subsequently, Europeans explored the possibility of providing enteral nutrition to the mentally ill patients also (Randall, 1990).

Further research work in this field led to the preparation of General-Category and disease-specific enteral foods based on defined or chemically defined ingredients. Later on, research was conducted on the feeding mode and feeding accessories. Subsequently, several clinical outcome studies on various formulae were reported mostly in USA, Canada, Europe, and Japan (Skipper, 1989; Parshad et al., 1993; Borlase et al., 1994; Hasse et al., 1995; Kaur, 2007). The important milestones in the development of enteral foods are presented in Table 2.2.

Table 2.2 Milestones in the development of enteral nutrition

Year	Introduced by	Purpose	Food	Route/device
Many years before Christ	Egyptian and Greek physicians	For preserving good health	Milk, wine, egg, beef, grain, and chicken broths	Rectal/silver tube
1598	European physicians	Nutritional support to patients	Milk, wine, egg, beef, grain, and chicken broths	Upper gut via nostrils to esophagus/hollow silver tube
1790	European physicians	To introduce stimulating substances into the stomach	Jellies, beaten eggs, sugar milk, medicines	Orogastric/hollow catheter
1810	US physicians	For feeding food and for gastric emptying	Jellies, beaten eggs, sugar milk, medicines	Orogastric/flexible rubber tube
1851	European physicians	For feeding mentally ill patients	Jellies, beaten eggs, sugar milk, medicines	Oral hypopharyngeal, stomach/pump with flexible tube
1910	European physicians	Nutritional support to patients	Milk, eggs, lacto-sugar	Orogastric upto duodenum/flexible tube with 10–12 g metal attached at distal end
1939	US physicians	Postoperative, patients, carcinoma, duodenal ulcer	Casein hydrolysates, dextrose peptonized milk, isotonic saline, vitamins	Nasogastric double lumen tube inserted up to small bowel

(continued)

Table 2.2 (continued)

Year	Introduced by	Purpose	Food	Route/device
1952	US Scientists	Controlled clinical studies on different category patients	Protein, cereal-rich hydrolysates, liver proteins, milk	Polystyrene tubes – different modes of feeding
1969 onward	US scientists	Complete nutritional support to seriously ill patients of various physiological conditions	Chemically defined diets	Nasogastric, gastrostomy

Adapted from Randall (1990)

Types of Enteral Foods

The concept of enteral nutrition started with blenderized foods, which subsequently underwent improvements into polymeric, modular, defined, and disease-specific enteral foods. Brief descriptions about the major types of enteral foods are presented in the next section.

Blenderized Formulae

Historically, enteral foods were prepared by blenderizing common foods such as rice gruel, milk, and meat broth, among others to make it suitable for oral or tube feeding (Harkness, 2002). Blenderized food satisfies the patient's emotional feeling of eating the normal food and permits incorporation of desirable food ingredients. They can be prepared at home or hospitals easily. Generally, these foods have high *dietary bulk* because of which patients' total food intake is restricted. Also, they may not be microbiologically safe (Campbell, 2006) and often require large-bore feeding tube. In addition, tube clogging occurs while feeding blenderized foods (Mobarhan and Trumbore, 1991; Mahan and Arlin, 1992).

Polymeric Formulae

Some of the drawbacks of the blenderized formulae are overcome by polymeric formulae. For preparing polymeric formula, the major food constituents, namely carbohydrates, proteins, and normal dietary lipids in pure form, are blended suitably. Very often soy polysaccharides are added as a source of dietary fiber. Generally, the polymeric formulae are lactose-free and their nonprotein calorie to nitrogen ratio ranges from 150 to 275 kcal:1 g nitrogen, and the osmolality varies from 300 to 700

mOsmol Kg^{-1}. An adaptation period of about 3 days may be needed to administer these formulae in full strength (Small and Heymsfield, 1983; Martin and Acosta, 1987; Kleibeuker and Boersma-Van, 1991).

Defined Formulae and Elemental Formulae

These enteral formulae are designed for easy digestion and absorption, and contain known quantities of chemically purified food constituents such as glucose, sucrose, maltodextrins, amino acids, peptides, and medium-chain triglycerides (MCT), monoglycerides, triglycerides, or a small amount of vegetable oil such as sunflower, safflower, or corn oil (Randall, 1984). Because of the chemical nature of the ingredients, these foods are generally unpalatable. So, to improve their palatability, very often, they are artificially flavored. In fact, the US National Aeronautics and Space Administration (NASA) scientist tested the feasibility of introducing the elemental formulae to the astronauts in order to avoid the problem of disposing human waste in outer space. Because of the poor flavor and aroma, NASA rejected these formulae (Winitz et al., 1965). Most of the elemental formulae are lactose-free and fiber-free and are usually designed for patients with compromised digestive or absorptive capabilities such as short bowel syndrome, radiation enteritis, chronic or acute pancreatitis, and some patients with Crohn's disease (Giaffer et al., 1990; Mobarhan and Trumbore, 1991).

Disease-Specific Formulae

Disease-specific enteral formulae were developed because patients with different physiological conditions or diseases needed different kinds of nutritional support. The major diseases and the suggested pattern of nutritional support to each of these diseases are given in Table 2.3. Besides essential macro- and micronutrients, the disease-specific enteral foods are usually enriched with conditionally essential nutrients such as glutamine (Furukawa et al., 1997; Kreymann et al., 2006), arginine (Barbul, 1990), taurine (Fischer et al., 1990; Laidlaw et al., 1990; ESPEN, 1995), and carnitine (Menon and Natraj 1984; Okuyama et al., 1997). A proper proportion of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is provided by blending suitably the fish oil in some of the formulae (Gerster, 1995; Kreymann et al., 2006).

Some of the disease-specific enteral foods are enriched with branched chain amino acids (BCAA) to provide about 40–50% of the protein calories. They reduce the putative false neurotransmitter effects and hepatic encephalopathy associated with excessive aromatic amino acid (AAA) in the brain. Moreover, in severely hypercatabolic conditions BCAA are utilized as a source of locally available energy in the skeletal muscle. The formulae enriched with BCAA help normalize the amino acid profile, improve protein synthesis, and maintain nitrogen balance

Table 2.3 Suggested pattern of specific nutritional support in some diseases

Disease/physiological conditions	Suggested pattern of nutritional support
Cancer	Calorie: 115–130% of resting energy expenditure (REE) for maintenance and additional 15% of REE for repletion. Protein: high protein (1.2–2.0 g kg^{-1} day^{-1}) preferably from animal source. Low fat and low residue, foods rich in vitamin C, thiamine, carotene, folic acid, iron, and zinc
Hepatic diseases	Calorie: high calorie (35–50 kcal kg^{-1} day^{-1}). Protein: moderate (0.5–1 g kg^{-1} day^{-1}), but rich in BCAA. Fat: moderate containing MCT. Foods rich in folate, thiamine, B_{12}, zinc, adequate potassium and iron. Sodium restricted (250–500 mg day^{-1} without diuretics, 1–2 g with diuretics)
Renal diseases	Calorie: 40 to 60 kcal/kg/day). Protein (without dialysis) low protein (0.6g to 0.8 g protein/kg/day) of biological value. Calcium 300 to 500 mg.) of high bioavailability. Sodium (up to 500 mg./day). potassium and fluid restriction depending upon the condition.
Pulmonary Disease	Low carbohydrate and high-fat diet. Energy: moderate level (25–35 kcal kg^{-1} day^{-1}) for maintenance and 35–45 kcal day^{-1} for repletion. Protein: 1.5–2 g kg^{-1} day^{-1}, high in phosphorus
Burns	Calorie: 25 kcal kg^{-1} usual body weight + 40 kcal × TBSA). Protein: 1.5–2.0 g kg^{-1} day^{-1} with a cal:N = 100:1, rich in arginine, glutamine, cystine, and BCAA. Fat: 15% of nonprotein calorie, rich in n-6:n-3 = 3.5:1. Vitamin C:1 g day^{-1}. Higher levels of all the vitamins and minerals than RDA. Inclusion of immunoenhancing components
Surgery/sepsis/trauma	Calorie: depends upon stress level (25–50 kcal kg^{-1} day^{-1}). Nonprotein calorie:N = 80–150:1. Protein: 1–2.5 g kg^{-1} day^{-1}. Fat: 25–30% of total kcal day^{-1}. Vitamins and minerals slightly higher than RDA
HIV/AIDS	Calorie: 50 kcal kg^{-1} day^{-1}. Protein: 1–1.2 g kg^{-1} day^{-1}. Cal:N = 100:1. Low fat rich in MCT. Enriched with immunomodulatory component
Patients of medicine and surgery	Calorie: 25–35 kcal kg^{-1} day^{-1}. Protein: 1–2 g kg^{-1} day^{-1} of high biological value. Fat: moderate level with n-6:n-3 = 6.1. Foods complete with all the essential vitamins, minerals conditionally essential nutrients, and shadow nutrients

Adapted from ASPEN (1995)

(Ferenci, 1996). These products are often recommended for patients under major metabolic stress (Cerra et al., 1983; Mobarhan et al., 1990; Ferando et al., 1995).

High-fat (up to 50%) enteral foods are advised to patients with chronic obstructive pulmonary disease (COPD) (Noller and Mobarhan, 1986). Such foods are reported to be well tolerated, even by debilitated elderly patients (Simko and Shoukry, 1989). The beneficial effects of high-fat formula for patients with abnormal glucose tolerance and COPD have been reported by Brandstetter et al. (1988).

Enteral formulae containing only essential amino acids and histidine have been formulated. These foods minimize the nitrogenous wastes to be handled by failing kidneys. They do not contain vitamins and minerals, and are of low fat content, but they contain trace amount of electrolytes. For example, Replena, an energy-rich (1,000 kcal per 356 mL.) and low-protein (14.9 g per 1,000 kcal) polymeric formula designed for oral and/or tube feeding of patients with chronic renal failure has been formulated (Mobarhan and Trumbore, 1991). Careful individualization and monitoring is necessary for long-term use of this kind of formulae.

Enteral foods supplemented with high proportion of Omega-3(n-3) polyunsaturated fatty acids, arginine, exogenous sources of purines and pyrimidines and glutamine have been developed (Babineau, 1994). These are known as immune-enhancing formulae and have therapeutic implications for metabolically stressed and immunocompromised patients.

Modular Formulae

Sometimes predetermined quantities of carbohydrates, proteins, and fat, either single or in combination are added to the food to increase the content of that particular nutrient in the food. Such foods are known as modular foods (Anderson and Kennedy, 1986). Information on some of the enteral foods currently being marketed is presented in Appendix A.

Criteria for Evaluation of Enteral Foods

In the last three to four decades, considerable research on enteral foods has been carried out and the specific requirements of this category of food have been largely defined (Talbot, 1990). It has been generally accepted that the energy density of enteral food should be 1–2 kcal mL^{-1} food, and the energy should be preferably derived from carbohydrates and fat. A liter of food should provide 25–50-g protein, and the ratio of calorie to nitrogen should be 80–100:1. The food can be liquid or ready-to-eat or ready-to-use powder form. The osmolality, complexity, fat contents, residue-forming characteristics, electrolytes, mineral contents, the sources of protein, fat, carbohydrate, and other nutrients, microbiological safety, and also the mode of compounding the ingredients are indicated in Table 2.4. The osmolality of

Table 2.4 Criteria for evaluation of enteral foods

1.	Calorie density	1–2 kcal mL^{-1}
2.	Protein content	25–50 g L^{-1}
	Protein calories	Standard <20%, high ≥ 20%
3.	Fat content	30–50 g L^{-1}
	Fat calories	Low 5–20%, very low <5%
4.	Residue (dietary fiber)	6–14 g L^{-1}
	Low residue	Up to 6 g L^{-1}
	Residue free	No fiber
5.	Osmolality (mOsmol kg^{-1})	350–550
	Isotonic	<350
	Moderately hypertonic	350–500
	Markedly hypertonic	550 and above
6.	Renal solute load	300–900 mOsmol L^{-1}
7.	Microbial safety	Free from pathogenic microflora
8.	Lactose content	May be considered for lactose intolerance patients
9.	Form	Ready-to-eat (liquid form) or ready-to-reconstitute powder form
10.	Route of administration	Oral, tube, and tube only
11.	Carbohydrate source	Corn starch, corn syrups, maltodextrin, sucrose, fructose, vegetables, glucose, oligosaccharides, suitably processed cereals and legumes
12.	Protein source	Proteins of animal origin – casein lactalbumin, egg, albumin, beef extract, amino acids, suitably processed or predigested vegetable proteins such as soy protein isolates, cereals and legume protein, whey protein concentrate, etc.
13.	Fat source	Long chain triglycerides (LCT), LCT + medium-chain triglycerides (MCT), structured triglycerides
14.	Vitamins and minerals content	Recommended dietary allowance delivered in about 2,000 kcal taurine, carnitine, nucleotide, conditionally
15.	Incorporation of special nutrients	For example, shadow nutrients like essential nutrients such as BCAA, BCKA, arginine, glutamic acid, EPA, DHA, etc. depending on the physiological condition
16.	Method of composition	Blenderizing to make it ready to use, dry blending of ingredients that can be reconstituted, processing ingredients to prepare dry or wet food

From Heimburger and Weinsier (1985)

enteral foods plays a major role in its acceptance and tolerance by the patients as hyperosmolar formulae generally cause diarrhea and may also act as iatrogenic (Cataldi-Betcher et al., 1983; Leguennec et al., 1983).

The composition of a few enteral foods in Appendix A indicates that maltodextrin, sucrose, and glucose are prominent sources of carbohydrates, and free amino acids, casein, and egg white are protein sources. Generally, soy oil, canola oil, corn oil, and fish oil form the lipid sources. The enteral foods produced and marketed by few pharmaceutical companies in developing countries like India are also similar to those marketed in developed countries. The calorie density of foods is mostly enhanced either by addition of fats or oil or by using highly soluble carbohydrates.

Maltodextrin or other sugars provide only empty calories and the protein hydrolysates or amino acids are generally unpalatable. Time and again, it has been observed that enteral foods akin to normal diet but of improved digestibility and texture perform better as nutritional support to the patients than elemental diet. However, the difficulties encountered with the preparation of nutrient-dense liquid foods from the normal food ingredients, which can flow easily through feeding tube of smallest permissible bore, have been the major constraints for the preparation of enteral foods akin to normal foods. From the foregoing discussion, it may be clear that the enteral nutritional support has a major role to play in the medical care of patients and geriatric subjects. Although considerable R&D work has been carried out in this area, till date, most of the enteral foods are prepared using chemically defined ingredients. Levine et al. (1985) reported that lack of understanding of food science and technology in medical professionals perhaps contributed to the more drug-like approach in medical food development. As discussed earlier, such foods generally are hyperosmolar, lack natural stimulants of the digestive tract, may alter gut microflora, and are less palatable. They are hygroscopic and require expensive packing. These chemically defined enteral foods are generally priced high and beyond the reach of most of the patients belonging to developing countries.

Hence, need was felt to develop enteral foods that can be afforded by majority of the patients and are akin to normal wholesome diet. To this end, it was hypothesized that the malted cereals and grain legumes, which possess improved bioavailability of nutrients and permit to prepare nutrient-dense and less viscous foods (Brandtzaeg et al., 1981), could be used for the preparation of enteral foods. In the next section, the nutritional and textural qualities of malted cereals and grain legumes and their suitability for utilization in the enteral formulations have been discussed.

Processing of the Ingredients for Development of Enteral Foods

Some of the food processing technologies could be gainfully exploited to develop enteral foods from natural sources. In this direction, an attempt was made to modify the textural and nutritional quality of natural food ingredients in order to make them suitable for enteral feeding. Careful manipulation of some food processing technologies such as malting, toasting, popping, fermentation, and spray drying among others offered such opportunities. Amazing scope of exploitation of some of these technologies is as follows.

Malting

Malting or controlled germination of cereals is one of the important food processing technologies used in the world, largely for brewing and to some extent for food purposes. Barley has the place of pride in malting and brewing industries

(Munck, 1981; Hoseney, 1994). Malting of wheat (Sethi and Bains, 1978; Suhasini and Malleshi, 1995), sorghum (Palmer, 1992), rice (Capanzana and Buckle, 1997, Chakravarty, 1997), and finger millet (Nout and Davis, 1982; Malleshi and Desikachar, 1986a) is also practiced in Europe, Africa, and Asia. On the other hand, sprouting of grain legumes for food is practiced globally (Khader and Venkat Rao, 1981; Vanderstoep, 1982; Kaur et al., 2007). Dried, sprouted legumes are termed as legume malt (Malleshi and Klopfenstein, 1996). Malting essentially involves steeping, germination, and kilning processes. Although these processes are independent of each other, the quality of malt depends on all these unit operations.

Steeping

Clean and viable seeds are steeped in excess water to facilitate hydration. Barley is usually steeped at 10–15˚C for 48–72 h to attain about 45% moisture. Generally, the optimum temperature of steeping for tropical cereals is 25C and the moisture content of steeped seeds ranges from 30 to 45˚ (Brookes et al., 1976). Replacing the steep water in the midcourse of steeping replenishes the oxygen. Adding hydrogen peroxide, sulfuric acid, lime, and formaldehyde to steep water is practiced to reduce the microbial growth, to overcome the dormancy and the water sensitivity of the seeds (Briggs, 1987; Doran and Briggs, 1993), and also to control mold growth that is manifested during germination (Swanston and Taylor, 1990). Addition of gibberellic acid at the terminal stages of steeping is generally followed to induce synthesis of α -amylase at the early stages of germination (Palmer, 1989). During steeping, the seed becomes metabolically active, and thus the process of germination initiates.

Germination

Germination is the most important stage of malting process wherein most of the biochemical and textural changes take place. Normally, the duration of germination varies from 2–5 days depending on the temperature of germination and the end uses of malt. The temperature and humidity of the germinating seeds are controlled to keep the sprouts moist either by spraying water or by using humidifiers. The sprouts are mixed gently to aerate and to dissipate the heat formed due to metabolic activity (Briggs, 1987). Some of the hydrolyzed products are utilized for the metabolic activity and a portion of them is also used for the growth of root and shoot (Hegazi et al., 1979). This causes the loss of some amount of endosperm reserves. So, to conserve the food reserves and the enzymes developed, the process of germination is terminated at an appropriate time by dehydrating the sprouts.

An array of enzymes, namely amylases, proteases, lipases, glucanases, and pento-sanases, are either synthesized or activated during germination (Enari and

Sopanen, 1986). Both α -amylases and β-amysases are activated during malting. α-amylases in cereal malt are endoenzymes that hydrolyze 1, 4 glycosidic linkages of the starch granules randomly (Knutson and Grove, 1992), whereas β -amysases are exoenzymes and cleave the glucosidic linkages of starch from the nonreducing end liberating maltose (Sopanen and Lauriere, 1989). The debranching enzymes of malt cleave α -1, 6 linkages of amylopectin and the limit dextrins (Roberts and Whelan, 1960). The pentosanases solubilize the cell walls of the starchy endosperm and facilitate the movement of amylases in the endosperm (McCleary et al., 1986).

Generally, the proteases develop during steeping and increase their activity by about 20-fold during germination. Activities of both proteinases and peptidases are increased during germination. Among the proteolytic enzymes, the proteinases (endopeptidases) account for about 90% of the total activity and are active at pH 5.4. The endopeptidases hydrolyze peptide bonds of protein molecules and the large polypeptides toward the interior of the chain and reduce their molecular weight. The activity of peptidases (exopeptidase) increases during germination either due to synthesis, or due to denaturation of inhibitors (Kruger and Preston, 1977). The protein bodies of scutellum and aleurone layers are degraded by the proteolytic enzymes and the amino acids liberated are mainly used in the synthesis of hydrolytic enzymes and other proteins needed for the growth of the seedling (Shutov and Vaintraub, 1987). As germination progresses, the protein matrix surrounding the starch granules gets partially digested, thus facilitating easy accessibility of the starch granules for amylases (MacGregor, 1980). The phytase developed on germination hydrolyzes phytate and enhances the mineral availability (Kikunaga et al., 1991).

Lipases and phospholipases are activated and released in the aleurone layer of the seed, and their activity increases sharply during germination (Urguhart et al., 1984). Nearly two-thirds of the lipases in germinating grain are present in the embryo and one-third in the aleurone layers (Anness, 1984). Although most of the cereals contain small amount of lipids, the increased lipase activity on germination has a bearing on keeping the quality of malt products.

Kilning

Generally, the germinating seeds are subjected to warm-air drying in the initial phase of kilning so as to bring down the moisture content to around 18%, followed by raising the air temperature to 70–80°C for a short time to induce the effect of curing and to dry the sprouts to about 6% moisture. During kilning, the material temperature is not raised beyond 70°C so as to cause minimum damage to the malt enzymes. However, in case of small-scale malting for food uses, the rootlets from the dried sprouts are separated and the green malt is kilned by contact heat in rotary heaters (cake roasters). During kilning, the reducing sugars and the free amino acids react, and thus resulting in slight browning of malt. This arrests the biological activity and produces the characteristic aroma of malt (Bathgate, 1973).

Nutritional Composition of Malted Cereals

Carbohydrates

Considerable increase in free sugars and decrease in starch content, especially the amylopectin, has been reported in cereals (MacLeod et al., 1953). Increases in glucose, sucrose, and maltose contents from 0.95 to 1.5, 9.5 to 15.9, and 1.3 to 2.4 mg g^{-1}, respectively, on 120-h germination of wheat were reported by Kruger and Matsuo (1982). Malleshi et al. (1986) reported several fold increase in low molecular weight sugars and degradation of oligosaccharides of raffinose series that causes flatulences on malting of millets. The solubility of cell walls (Prentice, 1976), partial digestion, and reduction in the rigidity of the starch molecule coupled with degradation of amylase inhibitors enhance the overall carbohydrate digestibility on malting (Finney, 1982; Marero et al., 1988). In case of legumes, the degradation of oligosaccharides and increase in carbohydrate digestibility have been reported (Ganesh Kumar and Venkatraman, 1976; Sumathi et al., 1995).

Proteins and Amino Acids

Generally, the storage proteins, namely the prolamins of cereals, are hydrolyzed by proteolytic enzymes on malting resulting in an increase in water-soluble proteins and free amino acids (Bhise et al., 1988). This has nutritional implications as prolamins are deficient in lysine, and the water-soluble proteins, namely albumins and globulins, are rich in lysine (Chavan and Kadam, 1989; Ariyama and Khan, 1990). An increase in lysine and tryptophan contents in cereals has been reported (Dalby and Tsai 1976; Wang and Fields, 1978). Finney (1982) observed an increase in lysine content initially up to 4 or 5 days of germination and then a decrease later in 12 varieties of wheat. Singh and Sosulski (1986) reported increases of 44% and 70% in lysine and tryptophan contents, respectively, on 8 days of germination of Glenlea wheat. Smith and Simpson (1983) observed an increase in lysine, alanine, aspartic acid, and glycine but decrease in cystine, glutamic acid, and proline in barley. Trypsin and other protease inhibitors are degraded during germination of legumes, thereby enhancing their protein digestibility (Jaya and Venkataraman, 1980). The nutrient composition including that of amino acids of native and malted cereals and legumes is given in Tables 2.5 and 2.6.

Vitamins and Minerals

A little increase in thiamine but a significant increase in riboflavin contents of cereals and grain legumes on sprouting has been observed by several researchers (Chavan and Kadam, 1989). A slight increase in ascorbic acid contents but consi-derable increase in riboflavin contents in sprouted seeds has been reported (Lorenz, 1980; Malleshi and Desikachar, 1986). Sprouted legumes are rich sources

Table 2.5 Nutrient composition (per 100 g) and amino acid contents (gram per 100-g protein) of some cereals and their malt

	Barley[a]		Wheat[b]		Rice		Finger millet[a]	
	N	M	N	M	N[c]	M[d]	N	M
Protein (g)	12.2	13.7	12.4	12.0	6.8	5.2	7.0	4.2
Fat (g)	1.7	1.9	1.8	1.7	0.5	0.8	1.5	1.1
Ash (g)	1.8	2.1	1.7	1.8	0.6	0.9	2.7	1.7
Crude fiber (g)	5.3	3.3	3.3	3.3	0.2	–	3.0	4.3
Thiamine (μg)	400.0	384.0	270.0	280.0	85.0	98.0	229.0	196.0
Riboflavin (μg)	200.0	310.0	70.0	120.0	60.0	120.0	93.0	159.0
Niacin (mg)	8.5	5.7	2.6	2.7	1.9	2.1	2.2	3.0
Ascorbic acid (mg)	1.0	1.8	0.7	1.1	–	–	0.1	1.0
Calcium (mg.)	26.0	22.0	53.4	60.1	12.0	9.0	372.0	346.0
Phosphorus (mg)	215.0	168.0	355.4	374.1	160.0	152.0	215.0	190.0
Aspsrtic acid	4.7	5.9	6.4	6.9	9.9	–	7.6	8.2
Threonine	2.1	3.3	3.3	3.3	4.5	–	4.3	4.3
Serine	4.3	4.2	5.3	5.1	4.8	–	5.5	5.2
Glutamic acid	26.4	23.4	29.7	28.4	17.0	–	21.0	18.5
Proline	11.7	11.1	7.9	8.1	4.4	–	6.4	6.3
Glycine	3.8	3.8	4.5	4.5	5.7	–	4.6	4.8
Alanine	3.9	4.5	4.3	4.4	4.3	–	7.0	7.1
Cystine	2.2	2.3	4.3	4.3	1.4	–	2.0	1.9
Valine	4.8	5.3	3.5	3.7	6.8	–	4.8	5.4
Methionine	2.0	1.4	1.8	1.7	2.9	–	3.4	3.3
Isoleusine	3.2	3.6	2.7	2.9	4.9	–	2.7	3.1
Leucine	7.1	7.4	6.8	6.9	8.4	–	8.8	9.1
Tyrosine	3.2	3.6	3.6	3.6	5.2	–	3.9	4.4
Phenylalanine	5.5	5.9	5.0	5.0	4.7	–	5.5	6.0
Histidine	2.4	2.4	3.2	3.2	2.0	–	3.1	3.2
Lysine	3.3	3.6	2.9	2.9	3.9	–	3.1	3.1
Arginine	4.7	4.7	4.7	4.7	8.3	–	4.4	4.2

N Native, *M* Malt
[a]Malleshi (1992)
[b]Suhasini and Malleshi (1995)
[c]Gopalan et al. (1989)
[d]Analyzed values

of B-group vitamins and are also known to contain some provitamin A and vitamin E (Pomeranz and Robbins, 1971; Voss and Piendl, 1978).

An increase in the ionizable iron from 4.1 to 7.7% and insoluble iron from 10.4 to 16.4% on germination of cereals has been reported by Prabhavathi and Rao (1979). Similarly, increases in acid extractability of calcium, phosphorus, iron, zinc, and copper from 48 to 69%, 36 to 48%, 36 to 50%, 42 to 50%, and 35 to 57%, respectively, were observed in malted wheat, malted green gram, and jaggery-based weaning foods (Gahlawat and Sehgal, 1993). A decrease in phytate phosphorus and an increase in hydrolyzed phytic acid during malting could be one of the contribu-ting factors for the enhanced availability of phosphorus and other minerals (Reddy et al., 1982; Wesley, 1993).

Table 2.6 Nutrient composition (per 100 g) and amino acid contents (gram per 100-g protein) of some cereals, legumes, and their malt

	Pearl Millet[a]		Sorghum[a]		Chick pea[b]		Mung bean[b]	
	N	M	N	M	N	M	N	M
Protein (g)	16.2	16.4	11.8	11.0	26.8	26.2	24.1	26.9
Fat (g)	5.3	5.0	2.3	2.7	6.0	7.3	1.0	1.5
Ash (g)	2.0	1.5	1.6	1.6	1.1	2.9	1.2	2.8
Crude fiber (g)	1.5	1.8	2.3	2.4	1.2	1.1	0.9	0.9
Thiamine (μg)	358.0	310.0	277.0	208.0	352.0	374.0	487.0	523.0
Riboflavin (μg)	196.0	204.0	107.0	153.0	181.0	186.0	163.0	256.0
Niacin (mg)	3.8	4.5	3.2	3.8	3.1	3.5	4.1	5.2
Ascorbic acid (mg)	0.4	1.7	0.1	0.7	1.3	2.8	1.4	3.5
Calcium (mg)	52.0	40.0	28.0	20.0	60.0	54.0	72.0	63.0
Phosphorus (mg)	265.0	222.0	200.0	182.0	330.0	298.0	395.0	350.0
Aspsrtic acid	9.3	9.3	7.6	7.2	12.9	12.8	13.0	13.2
Threonine	4.0	4.0	3.3	3.3	4.0	4.0	3.7	3.7
Serine	5.3	5.3	4.7	4.7	5.8	5.7	5.8	6.0
Glutamic acid	21.8	20.6	21.7	21.5	18.4	18.9	19.7	19.9
Proline	5.8	5.9	9.3	9.2	3.4	3.4	3.8	3.6
Glycine	3.2	3.0	3.2	2.9	4.4	4.4	4.0	3.9
Alanine	8.6	8.7	8.8	8.7	4.7	4.8	5.0	5.0
Cystine	1.6	1.9	1.9	2.1	0.9	0.9	0.5	0.5
Valine	4.7	5.2	3.3	3.7	3.1	3.2	3.8	3.9
Methionine	2.0	2.2	1.6	1.7	1.4	1.4	1.4	1.2
Isoleusine	3.3	3.6	2.6	2.9	3.0	3.0	3.1	3.2
Leucine	10.7	10.3	13.0	13.3	7.6	7.7	7.8	9.0
Tyrosine	3.3	3.6	4.1	4.4	3.2	3.2	3.3	3.3
Phenylalanine	5.1	5.3	5.1	5.4	6.1	6.0	6.2	6.2
Histidine	2.7	2.9	2.6	2.6	3.3	3.3	3.4	3.5
Lysine	2.4	2.4	1.8	1.5	6.9	6.5	6.9	6.8
Arginine	4.2	4.0	3.8	3.5	10.1	10.2	7.2	6.9

N Native, *M* Malt
[a]Malleshi (1992)
[b]Malleshi and Klopfenstein (1996)

Nutritious Foods Based on Malted Cereals and Legumes

The improvement in the bioavailability of nutrients of cereals and legumes by malting has been well recognized, and malted cereals and legumes have been used in the development of nutritious foods for different target groups. A high protein malt food containing 24.6% protein was prepared by blending malted wheat, low-fat groundnut flour, skim milk powder and fortified with vitamins and minerals. The malted food was nutritionally comparable with milk food (Chandrasekhara et al., 1957). Rajalakshmi and Ramakrishnan (1977) reported that a malted wheat and chickpea-based formulation was well accepted by the children and improved their nutritional status. Use of malted cereals for preparation of low-bulk and calorie-dense weaning and supplementary foods has been successfully attempted (Malleshi and

Desikachar, 1982; Marero et al., 1988; Kulkarni and Kulkarni, 1991; Gopaldas and Despande, 1992). Malted cereal-based foods were also well accepted and tolerated by aged women (Solanki, 1986). A biscuit formulation based on malted wheat and legumes was found to be a suitable nutrition supplement for the aged (Sumathi, 1997). Malted cereal flours, which are also known as amylase-rich food (ARF), are effectively used to reduce the viscosity of cereal-legume-based weaning foods (Mosha and Svanberg, 1983; Malleshi and Desikachar, 1988; Almeida-Dominguez et al., 1993; Wondimu and Malleshi, 1996). Beverages based on cereal malt and milk have also been prepared (Javalagi and Vaidehi, 1986; Singh and Bains, 1988).

As discussed earlier, cereal malt is a rich source of active carbohydrases. When the aqueous slurry of malt is heated, the enzymes get activated and simultaneously the starch gets gelatinized. This facilitates the enzymes to hydrolyze the starch to simple sugars and dextrins. In this process, the water-holding capacity of starch is reduced and a nutrient-dense and low-viscous food is formed.

The low viscosity of the food enables it to provide nutrition in a concentrated form to those who need liquid food including tube feeding. Malted cereals and legumes are nutritionally upgraded and texturally modified and could be used to prepare specialty foods such as enteral foods for patients. Malting is a simple and well-known technology, which can be adopted at household, community or industrial levels. Hence, studies were undertaken to prepare malt-based enteral foods.

Popping

Popping is one of the oldest and popular food processing technologies throughout the world. Popping of rice, wheat, corn, and other pseudo cereals such as amaranth seeds is a common method of preparing snack foods or supplementary foods (Baskaran et al., 1999). Popping and puffing are high-temperature short-time treatments (HTST) where the grains are subjected to 400–600° F temperature. The essential steps in popping are tempering the grains to 12–18% moisture content and then feeding the tempered grain to a popping device maintained at an appropriate temperature with continuous agitation. Finally, the popped grains are separated from the heat transfer media by sieving or vibrating. Traditionally, popping is carried out using edible oil as heat transfer media, or through contact heat in an iron or earthen pan.

Desirable Changes During Popping

Popping greatly improves the flavor and aroma of the grain, decreases and or destroys the surface bacteria present in the grain, and improves the starch digestibility. Popped grains are crunchy and light in texture offering a pleasant bite. Very high heat during popping may destroy certain amino acids like lysine and sulfur-containing amino acids (Tovar et al., 1989). However, improved popping method such as popping based on a fluid bed system (FBS) preserves these amino acids.

Toasting

Toasting is another dry heat processing of cereals and grain legumes. Toasting can be carried out on a stove top using a heavy-bottomed pan. No oil or grease is necessary, but a small quantity can be added to the pan to prevent the grain from sticking. Usually the pan is heated to 150–200˚ F and grains are toasted for 5–10 min. During toasting, the grain should be tossed and shaken to prevent it from burning. Oven toasting is another easy method of toasting grains. Nowadays, time–temperature-regulated grain toasters are available for pilot- and commercial-scale toasting.

Desirable Changes During Toasting

The surface microorganisms are often destroyed during toasting. A quick toasting of the grains adds flavor to the final dish. During toasting moisture levels are usually reduced, and thus enhancing the keeping of quality. When the flours from soybean and millet grains are toasted, lypolytic activities of certain enzymes are also reduced, thereby decreasing the spoilage of food by enzymatic rancidity.

Spray Drying

Spray drying is one of the most effective and widely used food processing technologies for dehydrating fluid foods such as milk, coffee, and egg powders, and is used as an encapsulation technique by the food and pharmaceutical industries.

During spray drying, the liquid food is generally preconcentrated by evaporation to reduce the water content. The concentrate is then introduced as a fine spray or mist into a tower or chamber with heated air. As the small droplets make contact with the heated air, they flash off their moisture, become small particles, and drop into the bottom of the tower. After this moisture is removed, the spray-dried powder must be collected aseptically and packaged in an airtight container, so that it does not reabsorb moisture from the air.

Principal components of the spray drying system include a high-pressure pump for introducing liquid into the tower, a device for atomizing the feed stream, a source of heated air with blower, a secondary collection vessel for removing dried food from the air stream, means for exhausting the moist air, and a facility for preconcentration. Atomizing devices are the distinguishing characteristic of spray drying. They provide a large surface area for exposure to drying forces (1 L = 12-billion particles = 300 sq. ft).

The exit air temperature is an important parameter to monitor during spray drying, because it responds readily to changes in the process and reflects the quality of the product. If the exit air temperature has a rapid fluctuation, then something

inside the system can be targeted as a problem area. This will ensure a constant awareness of product stability and equipment liability. Generally, processors want the air temperature high enough to yield desired moisture without heat damage. Altering feed flow rate or the inlet air temperature will control exit air temperature. If heat damage occurs before the product is completely dried, the particle size should be reduced, because smaller particles dry faster, and therefore they sustain less heat damage. By using a smaller orifice, increasing the atomizing pressure, and/or reducing the viscosity, particle size may be reduced.

Desirable Changes During Spray Drying

The combination of low-temperature and short-time (LTST) treatment employed during spray drying results in a high-quality product. Removal of water from food offers excellent protection against the most common causes of food spoilage.

Nutritional Quality of Spray-Dried Foods

Research has shown the lower viscosity of reconstituted spray-dried powder than that of the puree at the same solid concentration (Grabowski et al., 2008). Reduced viscosity in foods is a desirable quality, because a food with low viscosity delivers more energy per unit consumption than the food with high viscosity (Chakravarty, 2001). Spray-dried foods have better protein digestibility than the roller-dried foods. Moreover, LTST treatment during spray drying favors retention of other heat-sensitive nutrients such as some vitamins and amino acids. Because losses of nutrients are minimum in spray-dried foods, most of the specialty foods such as baby foods, milk foods, and health foods among others are generally spray-dried.

Ingredients of the Enteral Foods

Cereal malts (barley, rice, and finger millet), popped grain amaranth, mung bean malt, defatted soy, milk, egg, soy oil, and fish oil were the major ingredients identified for the enteral food. Barley is known for its therapeutic and diuretic properties besides being a good source of carbohydrates and soluble dietary fiber. Rice protein and carbohydrates are easily digestible, whereas finger millet is a rich source of sulfur-containing amino acids and calcium. Grain amaranth provides lysine-rich cereal protein and also dietary carbohydrates. Mung bean is a very good source of protein containing BCAA and its proteins complement well with cereal proteins. Defatted soy provides protein as well as polysaccharides (dietary fiber). Milk and egg are selected as sources of proteins with high biological value. Soy and fish oils

are chosen as sources of lipid because they can be blended meticulously to obtain a desirable ratio between n-3, and n-6 fatty acids. Coconut oil is an excellent source of MCT that does not need the multienzymes system to digest and could be an alternative source of oil for a patient having difficulty in fat digestion and absorption. Hence, a small amount of coconut oil has also been used as one of the ingredients of enteral foods.

The next chapter explains about the preparation of the malt flour.

Chapter 3
Preparation of Enzyme-Rich Fiber-Regulated Flour

Introduction

Cereal malt is a primary product and requires further processing in different ways for various end uses. During germination, the endosperm undergoes physiological, biochemical, and textural modifications (MacLeod, 1967; Briggs and McDonald, 1983; Oh and Briggs 1989; Sa and Palmer, 2004; Go malting, 2006; Priest and Stewart, 2006). The seed coat or husk of cereal malt, specifically that of rice and barley, is nonedible and contains high amount of silica. Bran from other cereals also contains some of the antinutritional factors such as phytates, oxalic acid, and polyphenols. Hence, the husk and bran need to be separated from the malted seed to prepare edible malt flour. This has special significance specifically when the malt is intended to use for the enteral foods. Generally, rice (paddy or rough rice) is first dehusked in a rice sheller, and then the brown rice is debranned to a desired degree in rice pearlers or polishers (Wadsworth, 1991). On the other hand, the seed coat of barley is separated in specially designed pearlers (Peterson, 1994). Finger millet has soft endosperm covered with tough bran, and pearling or polishing of millet is not possible because its endosperm pulverizes during pearling. Hence, refined flour from millet is prepared by adapting moistening, grinding, and sieving techniques (Desikachar, 1980; Malleshi and Desilachar, 1981a; Reddy et al., 2004).

Malting modifies the endosperm texture and renders it highly friable, thereby altering its milling characteristics (Henry and Cowe, 1990; Hoseney, 1994; Priest and Stewart, 2006). A little impact or friction on malted grain causes breakage and even pulverizes the modified endosperm along with bran. Moreover, the fully modified (enzyme-rich) portion of the endosperm is softer than its less modified portion. Hence, the enzyme-rich portion of the endosperm easily pulverizes and mixes with husk and bran fractions during milling. Heat generation (that occurs generally during milling) while milling the malt should be minimum to prevent possible heat damage to the enzymes. These factors indicate that for milling of malted cereals the conventional cereal milling technologies require modifications.

Malting of barley has been studied, and various unit operations involved in industrial malting have been standardized (Hudson, 1986; Doran and Briggs, 1993; Hickenbottom, 1996; Agu and Palmer, 1997; Laitila et al., 2002). But information

M. Kaur, *Medical Foods from Natural Sources,*
DOI: 10.1007/978-0-387-79378-8_3,

on malting of rice is scanty although the limited literature on rice malt reveals that the germinated rice possesses both liquefying and saccharifying enzymes (Aniche and Okafor, 1989; Egwim and Oloyede, 2006). Because these liquefying and saccharifying enzymes could predigest the complex carbohydrates and protein molecules to their simpler form, malted rice could be a good cereal base for enteral foods. Hence, the chapter explains the studies that were carried out on malting of rice and milling of malted rice to prepare enzyme-rich fiber-regulated flour. Similar studies were conducted on barley to prepare enzyme-rich and fiber-regulated flour suitable for incorporation in enteral food development.

Materials and Methods

Rice Malting and Milling of Malted Rice

Rice Malting

A few popular Indica and Japonica rice (*Oryza sativa*) varieties varying in amylose content were selected for studying the malting characteristics. The samples were cleaned off extraneous matter and damaged and immature kernels. The seeds were steeped in excess distilled water for 24 h, changing the steep water once in 8 h and germinated on spongy cloth in a BOD incubator maintained at 25C ± 2°C. Germination was continued up to 72 h, and the sprouted seeds were sorted out, dried in air oven at 50 ± 2°C, and the rootlets were separated by gentle brushing. The derooted malt was dehusked by hand, and the brown rice malt was pulverized in a UD cyclone sample mill (UD Corporation Colorado, USA), and the whole meal was assayed for amylase activity following the method of Bernfeld (1955). The methodology followed for assay of amylase activity is described next.

Assay of Amylase Activity

The malt amylases were extracted in acetate buffer, and the reducing sugars released by the action of the enzymes on soluble starch were estimated to assay the amylase activity. Because both β-amylases (liquefying) and β-amylases (saccharifying) are extracted in acetate buffer of pH 4.8, the term *amylase activity* is used throughout the study to express the additive activities of both the amylases.

Reagents for the assay: (1) Dinitrosalicylic acid (DNS): One gram of 3.5 dinitro salicylic acid (Loba Chemie, Bombay, India) was dissolved in 80 mL of 30% sodium potassium tartrate and 20 mL of 2N sodium hydroxide. (2) Acetate buffer (0.05 M, pH 4.8): sodium acetate (4.1 g anhydrous or 6.8 g hydrous) was dissolved in 100-mL water and to that 3 mL of glacial acetic acid was added (1 mL of 1% $CaC1_2$ solution was also added as a source Ca^{++} as enzyme activator), and the

contents were diluted to 1 L. (3) Soluble starch: One gram of soluble starch (Merck, Bombay, India) was dispersed in 50-mL water and the same was added to 50-mL boiling water and stirred well (freshly prepared for each assay). (4) *Standard maltose:* Two-hundred milligrams of maltose (Sigma, St. Louis, USA) was dissolved in water and volume was made up to 100 mL with water.

Enzyme extraction: A known quantity (50–100 mg) of the malt flour was suspended in 20 mL of 0.05 M acetate buffer for 30 min at room temperature (20–25°C) and the enzymes were extracted by constant shaking. The contents were filtered through Whatman no.1 filter paper, and the filtrate was used for the assay of amylase activity.

Assay procedure: To 0.25–1 mL of enzyme extract (depending upon the nature of sample) in a test tube, 1 mL of soluble starch was added and incubated at 37°C for 30 min. The enzyme activity was inhibited by addition of 2-mL DNS reagent and the volume made up to 4 mL with distilled water. The contents were heated for 5 min in boiling water bath, cooled, and diluted to 20 mL with distilled water. The color developed was read at 540 nm. Sample blank was prepared by adding the DNS reagent to the enzyme extract to inhibit the enzyme activity (DNS reagent + starch + extract), and a reagent blank was prepared without adding the enzyme extract. The activity was expressed as milligrams of maltose released by the action of amylases extracted from 1 g of malt on 1% soluble starch incubated for 30 min at 37°C expressed in maltose unit (Mu).

$$\text{Amylase activity (Mu)} = \{(A - B)/C\} \times Y \times \text{dilution factor}$$

where A = optical density (OD) of the experimental sample, B = OD of sample blank, C = OD of standard maltose, and Y = milligram of standard maltose present in maltose solution, corresponding to OD for standard maltose.

To find out the influence of the duration of germination on elaboration of amylase activity, *Intan* and *IR 20* varieties were germinated for up to 8 days. Samples were withdrawn on every 24 h, dried, and their amylase activity was assessed. The results of the earlier experiments indicated that germination of paddy for 3 days was necessary for preparation of malt of adequate amylase activity. Among the rice varieties evaluated, *Intan* variety exhibited good malting characteristics (Figs. 3.1 and 3.2). It is easily available and is priced moderately. In addition, its malt has pleasant aroma and appealing color. Because of these qualities, *Intan* rice variety was used for bulk malting and preparation of malt flour.

Bulk Malting

Intan rice of over 95% germinating capacity, procured form Karnataka State Seeds Corporation, Mysore, India, was used for bulk malting. The seeds were washed and steeped in potable water for 24 h. The steeped water was replenished at about 8-h intervals and the floatings were discarded. The steeped seeds were spread (1-in. bed thickness) on moist jute bag, covered with another moist jute bag, and were allowed

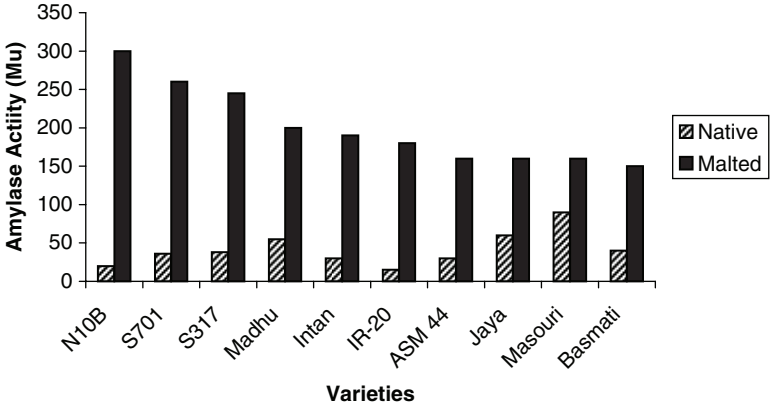


Fig. 3.1 Amylase activities of selected native and malted rice varieties

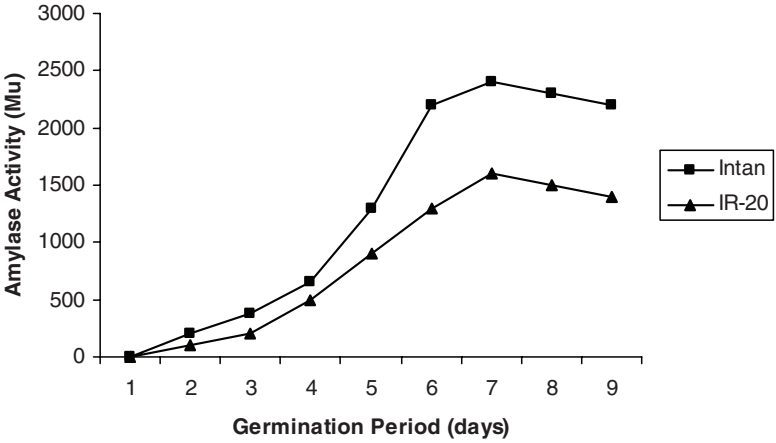


Fig. 3.2 Amylase activities of Intan and IR20 rice varieties

to germinate at ambient temperature (25 ± 3°C) for 72 h. Water was sprayed periodically to maintain the moisture content of the seeds and to facilitate even germination. The germinating seeds were gently mixed to prevent interlocking of the rootlets, provide aeration, remove carbon dioxide, and dissipate heat. The sprouts were air-dried (sun drying can also be followed) at 50 ± 5°C to about 12% moisture level. The rootlets were removed by gentle brushing in a machine (Westrupashed type LAH no. 240572 KAMAS, Kvarnmaskiner, Sweden). The derooted green malt was kilned in a grain roaster (Bharat Electricals, Bangalore, India) maintained at about 60°C by contact heat till the malt developed characteristic aroma.

Milling of Malted Rice

The exploratory milling studies on malted rice were carried out in rice huller (Ostad Engineering Co, Nagpur, India), rice sheller (Sri Ganesh Engineering Works, Chennai, India), cone polisher (M/s Dandekars, Chennai, India), horizontal emery pearler (Binny Modern Rice Mills, Mumbai, India), and McGill polisher (Burrows Equipment Co., Evaston, IL). The brief description of the machinery and characteristics of malted rice milled in the machinery are given in Table 3.1. Milling the rice malt in the rice huller caused multiple breakages of malt, and its softer portion got pulverized and admixed with husk and the same was not recoverable. Dehusking in

Table 3.1 Characteristics of malted rice milled in cereal milling machinery

(1) *Name of the mill:* Rice huller (Engelberg huller)

Brief description of the machinery: Consists of an iron-ribbed cylinder mounted on a rotating shaft fitted in a cylindrical housing. Friction between the grains and the shaft of the huller scrapes the husk and bran in one operation

Milling characteristics observed: Almost 100% breakage of malt, pulverization of softer portion of malt to a larger extent, and admixing with husk and bran. The malt is very poor in enzymes

(2) *Name of the mill:* Centrifugal sheller (for dehusking only)

Brief description of the machinery: Consists of high-speed rotating discs surrounded by a statio-nary rubber rim. The paddy fed is thrown by the rotating disc against the rubber rim with much force. The husk gets cracked by impact and the paddy is dehusked

Milling characteristics observed: High breakage but dehusking takes place effectively. Softer portion of the malt becomes powered and admixes with husk

(3) *Name of the mill:* Rubber roll sheller (for dehusking only)

Brief description of the machinery: Consists of two rubber rolls rotating in opposite directions at different speeds, which subjects the paddy grains falling between the rolls to a shearing action that strips off the husk

Milling characteristics observed: Moderate breakage but no pulverization and loss of malt. Dehusking paddy in a phasewise manner after separating the unhusked paddy can be continued until 100% shelling is achieved

(4) *Name of the mill:* Rice huller (for debranning only)

Brief description of the machinery: Consists of an iron-ribbed cylinder mounted on a rotating shaft fitted in a cylindrical housing. Friction between the grains and the shaft of the huller scrapes the husk and bran in one operation

Milling characteristics observed: Powdering of a considerable portion of malt and mixing the same with bran occurs. The dehusked rice malt is a poor source of enzymes

(5) *Name of the mill:* Cone polisher (for debranning only)

Brief description of the machinery: Consists of truncated cone, covered with emery, which rotates inside a wire screen (perforated sheet). The brown rice rotates around the cone and moves down between the cone and the screen. Because of the abrasion, the bran peels off and separates through the screen

Milling characteristics observed: Powdering of softer and enzyme-rich portion of malt occurs and the same gets mixed with bran. The debranned rice malt is a poor source of enzymes

(6) *Name of the mill:* Horizontal abrasive polisher (for debranning only)

(continued)

Table 3.1 (continued)

Brief description of the machinery: Consists of abrasive (carborundum) roll or disc attached to a steel shaft, rotating in a cylindrical, perforated metallic screen mounted horizontally. Abrasion between the role and the perforated screen peels off the bran layer

Milling characteristics observed: Almost same as that recorded for the cone polisher

(7) *Name of the mill:* Mini grain mill (for grinding and bran separation)

Brief description of the machinery: Consists of a plate mill having two serrated iron plates, one of which is stationary and the other is rotating. The material gets crushed and powdered and falls on a sifter, wherein the fine flour passes through, and coarse bran remains on top of the sieve

Milling characteristics observed: Major portion of the bran gets pulverized to fine particles and mixes with the malt flour

(8) *Name of the mill:* Experimental roller flour mill (for grinding and bran separation)

Brief description of the machinery: Consists of coupled pair of rolls divided into three break rolls (B_1, B_2, B_3) and three reduction rolls (C_1, C_2, C_3), amounting to six passages. The gap between the rolls are adjustable. The break passages are individually corrugated. The milled material from the first roll is sieved and the tailings are pneumatically transferred to the next roll. Because of divided sifter compartments, the individual milled fraction can be collected separately

Milling characteristics observed: Very effective for preparation of malt flour free from bran. Enables to prepare flour fractions of different amylase activities and dietary fiber content

centrifugal sheller also resulted in extensive breakage of malt. On the other hand, shelling the malt in rubber roll sheller (Millmore, Chennai, India) produced dehusked malt with no significant damage to the endosperm. Hence, rice malt was dehusked in rubber roll sheller to 100% shelling, and the debranning of brown rice malt was explored in different rice polishing machinery. Debranning the brown rice malt in rice huller, or in emery-coated horizontal and cone polishers pulverized the softer portion of the malt endosperm. On the other hand, exploratory studies on milling of brown rice malt in roller flour mill were promising for preparation of malt flour free from bran. Hence, detailed milling trials of brown rice malt were conducted in roller flour mill (Automatic laboratory mill, Type MLU 202, Buhler).

The effect of moist conditioning on the milling characteristics of malted rice was investigated. The rice malt with husk (malted paddy) was moistened with 2–10% additional water, tempered for 10–15 min, and was milled in the experimental roller flour mill at a feed rate of about 5 kg h^{-1}. The individual milling fractions and the straight-run flour were examined microscopically, and it was observed that the flour fractions were contaminated with pulverized husk and tegmen, although moistening reduced the pulverizability of husk. On the other hand, milling of the dehusked rice malt (brown rice malt) after moist conditioning was promising because the malt flour was almost free from pulverized bran fractions. Hence, the brown rice malt was sprayed with 2, 4, 6, and 8% additional water, tempered for about 10 min in a tempering bin and milled in the experimental roller flour mill. The roll gaps were suitably adjusted to minimize the pulverizability of bran (Table 3.2). The milling fractions were pooled together, equilibrated and the yield of flour, shorts, and bran was recorded.

To determine the seed coat (pulverized husk and bran) content of the malt flour from each milling trial, 10-g flour was dispersed in 100-mL water, and the contents were cooked in a water bath with constant stirring. While cooking the slurry, 0.5 mL of amylase (sigma, bacterial source, 5×10^6 units mL^{-1}) was added to supplement the

Table 3.2 Roll gap and sifter particulars of experimental roller flour mill

Rolls	Roll gaps (mm)	Sifter details: Dimensions (cm) I lay	II lay	III lay	Sizes (μ) I lay	II lay	III lay
Rice							
B_1	0.495	9.5 × 69.0	9.6 × 64.5	–	720	150	–
B_2	–	10.0 × 69.0	10.0 × 64.5	–	590	129	–
B_3	0.035	9.5 × 69.0	9.5 × 64.5	–	525	129	–
C_1	0.104	9.5 × 61.0	9.5 × 64.5	–	150	183	–
C_2	–	9.0 × 50.0	10.0 × 64.5	10.0 × 64.5	525	129	183
C_3	0.018	9.5 × 61.0	9.5 × 64.5	–	129	183	–
Barley							
B_1	0.465	9.6 × 69.0	9.6 × 64.5	–	720	150	–
B_2	–	10.0 × 69.0	10.0 × 64.5	–	590	129	–
B_3	0.035	9.5 × 69.0	9.5 × 64.5	–	525	129	–
C_1	0.065	9.5 × 60.5	9.5 × 64.5	–	183	183	–
C_2	–	9.0 × 50.0	10.0 × 64.5	10.0 × 64.5	525	183	183
C_3	0.035	9.5 × 60.5	9.5 × 64.5	–	183	183	–

B1, B2, B3 and C1, C2, C3 represent break rolls and reduction rolls 1, 2, and 3, respectively

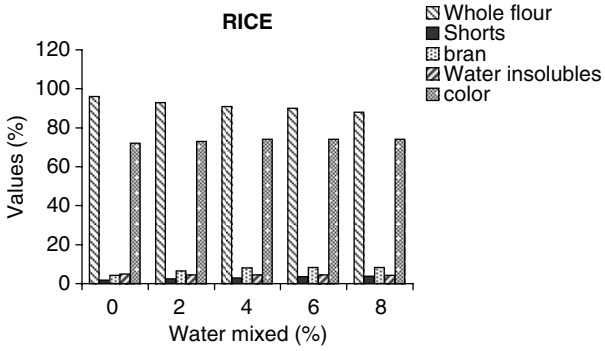


Fig. 3.3 The influence of moist conditioning and milling of malted rice and barley on the yield of mill fractions, water insolubles, and color of flour

malt enzymes for facilitating complete hydrolysis of starch. The starch hydrolysis was ensured by iodine blue test. The contents were sieved through a 150-mesh sieve (BSS), and the residue was washed repeatedly to free it from the solubles. The residue was collected over a weighed Whatman no.1 filter paper; dried, and weighed to determine the water-insoluble husk and bran contents. The color of the malt flour was also measured in a double-beam UV visible spectrophotometer with integrating sphere model (UV 2100/3100, Shimadzu, Japan) against $BaSO_4$ taken as 100% reflectance.

The influence of moisture on the yield and the quality of the flour, specially with respect to the bran content and the color of the flour, indicated that addition of 2.5% moisture and conditioning for about 10 min were suitable pretreatments needed to obtain malt flour of desirable qualities (Fig. 3.3). Based on

these milling experiments, a flow chart for milling of malted rice was developed (Fig. 3.4), and the flow chart was used for pilot scale milling of malted rice (Malleshi et al., 1996).

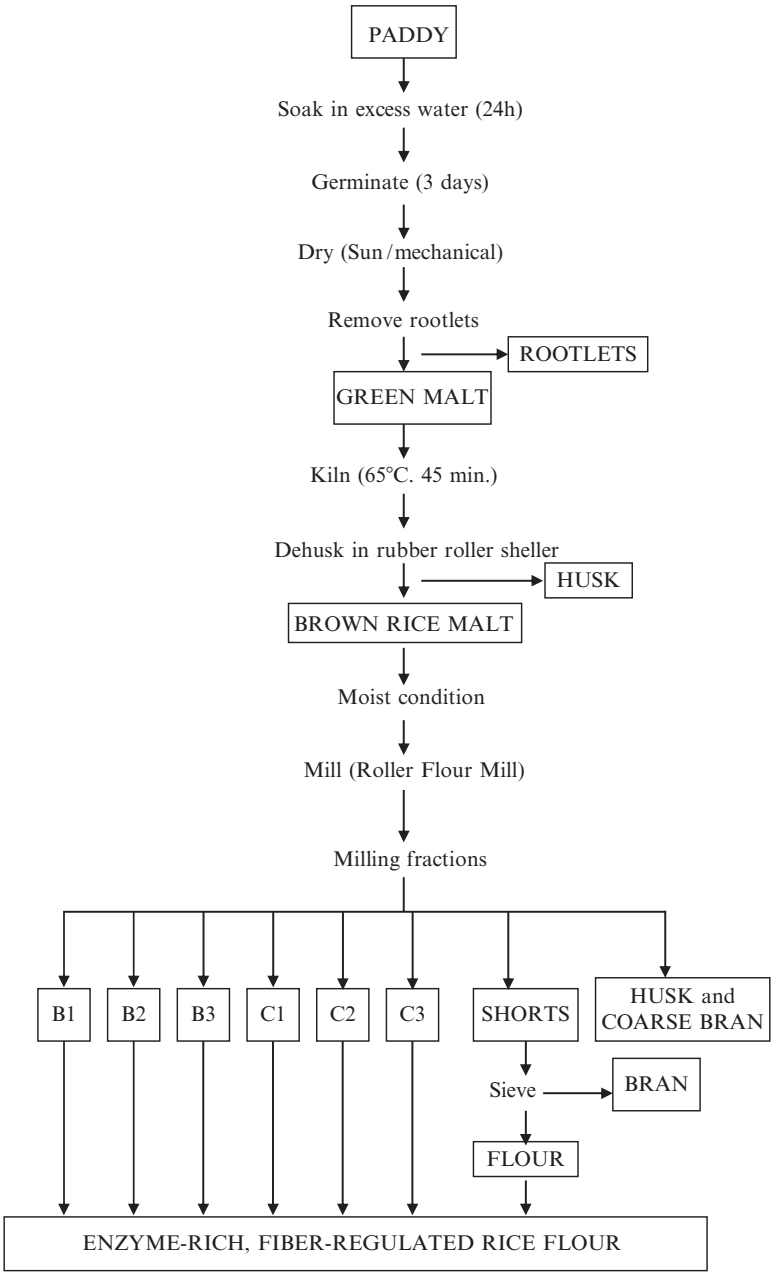


Fig. 3.4 Flow chart of malting of rice and milling of malted rice in roller flour mill

During the pilot scale (25-kg batch) milling, the individual mill fractions from break roll (B_1, B_2, B_3), reduction roll (C_1, C_2, C_3), and the bran duster through (BDT) fractions were collected individually. In a separate 5-kg batch milling trial, the flour fractions were pooled to obtain straight-run flour (whole flour). The proximate compositions of individual fractions and the whole flour were determined as per AACC (1983).

Soluble, insoluble, and total dietary fiber contents were estimated as per the method suggested by Asp et al. (1983). The color measurement and the assay of amylase activity were carried out as described earlier.

The viscosity of the fractions and the whole meal was determined as follows: cold-water slurry containing 5–50% solids was heated in a water bath with constant stirring for about 30 min to boiling, followed by direct boiling for 1 min on a hot plate to ensure complete cooking. The cooked mass was cooled to room temperature (25°C), and the viscosity was measured in a Brookfield viscometer (LVT model, Stoughton, USA) at 100 rpm using appropriate spindles, depending on the slurry viscosity. The viscosity is reported in centipoise units (cP).

Barley Malting and Milling of Malted Barley

Barley (*Hordeum vulgare*) purchased from Chandigarh (India) was cleaned off extraneous matter, steeped for 24 h, and germinated for 3 days at room temperature (20–25°C). The sprouts were dried, derooted, and kilned following the methodo-logy described for paddy. The amylase activity and viscosity of the barley malt prepared at the laboratory were compared with the food grade malt procured from The Malt Company (India) Ltd, Gurgaon, India. The quality characteristics of both the malts were comparable. Hence, malt from The Malt Company (India) was obtained in bulk and processed for enteral food formulations. The preliminary milling trials, specifically decortication trials of the barley malt in barley pearler and other cereal milling machinery, were not encouraging because a considerable portion of the malt was lost along with the seed coat during milling. However, moist-conditioning the malt and milling in roller flour mill minimized the pulverizability of seed coat. Hence, experiments were conducted to optimize the milling conditions in experimental roller flour mill. Five-kilogram batches of malt with initial moisture level of 6.2% were moistened with 2, 4, 6, and 8% additional water, tempered for about 10 min, and milled in experimental roller flour mill following the method described for rice malt. The quality of flour in terms of color and the seed coat content indicated that moistening the malt with 5% additional water and tempering for about 10 min was an optimal condition to obtain fiber-regulated and enzyme-rich barley malt flour (Fig. 3.3). After several trials, flow chart for milling of barley malt flour with desirable fiber content and enhanced enzyme activity was also developed (Fig. 3.5). Milling of barley malt on an industrial scale was carried out by spraying the malt with 5% additional water, tempering for a few minutes,

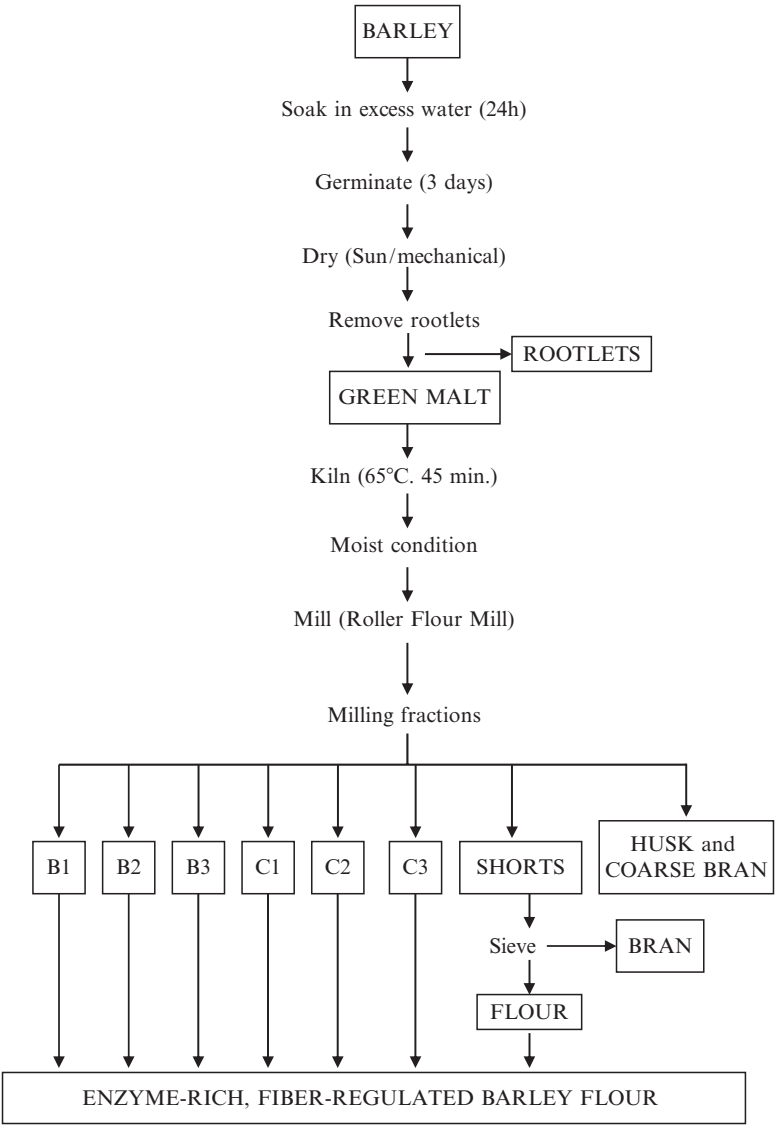


Fig. 3.5 Flow chart of malting of barley and milling of malted barley in roller flour mill

and milling in a commercial roller flour mill. The individual milling fractions and the straight-run flour (whole flour) were analyzed for proximate composition, dietary fiber contents, amylase activity, viscosity, and color following the methods as described earlier.

Finger Millet Malting and Milling

The *Indaf* variety of finger millet (*Eleusine coracana*), procured from Karnataka Agro Seeds Corporation, Mysore, was steeped in water for 16 h, germinated for 48 h, dried to about 14% moisture level, derooted, and kilned at about 65°C. The kilned malt was sprayed with 7% additional water and milled in a hammer mill (Apex comminuting mill). The malt was sieved through a 100-mesh sieve to prepare finger millet malt flour, following the method of Malleshi and Desikachar (1981a).

Mung Bean Malting and Milling

Uniform, bold sized seeds of mung bean (*Phaseolus aureus*) having over 95% germination capacity were procured from Indian market (Mysore, India). The grains were cleaned off extraneous matter, washed, soaked in excess water, and germinated for 24 h. The germinated grains were dried to around 12% moisture content. The dried sprouts were dehusked and derooted simultaneously in a rice huller. The husk and the rootlets were aspirated off and the spilt malt was kilned in a cake roaster at 80 ± 5°C till desirable aroma developed (Malleshi and Desidachr, 1982). The malt was pulverized in a plate mill and passed through a 100-mesh sieve (BSS) to obtain fine mung bean malt flour.

Grain Amaranth Popping and Milling

Grain amaranth (*Amaranthus cruentus*) white seeds were purchased form the local market, sprinkled with dilute butter milk to raise the moisture content to around 17%, tempered for about 4 h in a closed container and popped over an iron pan heated by wood fiber. The seeds were agitated continuously during popping to prevent surface charring and were removed from the pan as soon as popping was completed (Malleshi and Desikachar, 1981b). The unpopped seeds were separated by screening through a sieve. The popped amaranth was powdered in a hammer mill and sieved through a 100-mesh sieve (BSS) and the through (100-mesh popped amaranth flour) was used for the enteral food formulation.

The cooked paste viscosity of native and malted finger millet, mung bean, and popped amaranth at different slurry concentrations was determined in the Brookfield viscometer as described earlier.

Scanning Electron Microscopy

Native and 72-h malted rice and barley, 48-h malted finger millet, 24-h malted mung bean, and popped amaranth were cut open to expose the endosperm. The samples

were mounted on stubs with double-side scotch tape and were coated with gold (about 100°A) in a KSE 2 AM Evaporation Seevac gold sputter. The samples were scanned in Hitachi Scanning Electron Microscope (SEM) and the selective portions, mainly depicting malt modification, were photographed.

Results and Discussion

Wide variations among the rice varieties with respect to amylase activity of malts were observed. The malt from *N 10B* and *Basmati* variety exhibited the highest (280 Mu) and the lowest (145 Mu) amylase activity, respectively (Fig. 3.1). Although the native *Masuori* had noticeable level of amylase activity, its malt was a poor source of amylases. The malts form *Intan*, *IR 20*, *Madhu*, and *Jaya*, the popular varieties in the Southern region of India, exhibited amylase activity of intermediate levels. The amylose content of the varieties ranged from 5.1 to 29.4%.

The elaboration of amylase activity in *Intan* and *IR 20* varieties as a function of germination time is presented in Fig. 3.2. In both the varieties, the development of amylases was very slow up to 2 days of germination after which there was a steep rise up to 7 days, which subsequently decreased to a little as germination progressed. In the two varieties, the activity was higher in *Intan* at all comparative periods of germination. *Intan* malt had highly desirable aroma and acceptable taste and the dehusked malt was brighter in color (probably due to low bran pigmentation) than malt from *IR 20*. Therefore, malt from *Intan* variety was used for the enteral food formulations. Detailed investigations probing the reasons for varietal variation on the malting quality were not undertaken because this study was mainly aimed at identifying a suitable variety for preparing malt for enteral foods.

A limited number of reports on amylase of rice indicates that ungerminated rice is a poor source of amylases. Germination enhances its amylase activity and germinated rice contains both saccharifying (β-amylase) and liquefying (α-amylase) enzymes (Karmarkar and patwardhan, 1931; Kneen, 1944; Smith et. al., 1987; Egwim and Oloyede, 2006). Murata et al. (1968) reported that rapid breakdown of starch reserved in endosperm starts after 4 days of germination and results in formation of sucrose, glucose, and maltodisaccharides. Matsui et al. (1977) characterized the α-amylases of rice and reported that the molecular weight of α-amylase is about 53,000 with isoelectric point of 5 and optimum pH 5.5–6.5. Okamoto and Akazawa (1979) showed that the rice amylase was initially concentrated in the epithelium septum between the scutellum and endosperm but, later on germination, it was synthesized in the aleurone layer also. Palmiano and Juliano (1972) and later Capanzna and Malleshi (1989) studied the biochemical changes on malting of rice and reported that starch breakdown occurred on progressive germination, showing concomitant increase in low molecular weight sugars. A slight reduction in protein and a significant breakdown of phytate phosphorus have been reported on malting of rice (Kikunaga et al., 1991). Capanazana and Buckle (1997) identified the optimal malting conditions for rice following response surface

methodology. Later, Hammond and Ayernor (2001) reported that germinating rice for 10 days and preparing malt from germinated rice seeds with plumule and radicle could give rice malt a high diastatic power for the hydrolysis of starch in the production of sugar syrup.

Although varietal variations in malting characteristics of barley (Allison, 1986; Bhatty, 1996; Briennan et al., 1997; Kroth et al., 2005), wheat (Weseley, 1993), sorghum (Kumar et al. 1992), and finger millet (Malleshi and Desikachar, 1979; Gimbi and Kitabatake, 2002) have been reported, such information on rice is scanty.

Steeping rice for 24 h enhanced its moisture content to 33%, and uniform germination was observed. Drying the sprouts to about 14% moisture facilitated easy removal of rootlets, and kilning the green malt improved the taste and aroma of the rice malt.

As described earlier, by dehusking the rice malt in rubber roll sheller, it was possible to obtain decorticated malt without loss of enzyme-rich endosperm, although a few kernels were broken into two to three pieces. Dehusking or shelling initially to about 50% of paddy instead of 100% was found to be advantageous because the breakage was further minimized and the loss of the modified endosperm was avoided. Hence, phasewise dehusking or shelling of the malt was done in rubber roll sheller and the brown rice malt was milled in roller flour mill (Table 3.3). Many investigators have reported that milling of rice in two phases could either prevent or lessen the breakage in milled rice (Mathew and Spadaro, 1974; Nishita and Bean, 1982; Afzalinia et al., 2004).

The yield and composition of milled flour fractions obtained by milling brown rice malt moistened with 2.5% additional water and milled in roller flour mill are presented in Table 3.4. The yield of the flour from reduction roll (C_1 and C_2)

Table 3.3 Yield and amylase activity of rice malt milled in different cereal milling machinery

Mill	Material milled	Yield of dehusked and debranned malt (%)	Nature of milled malt	Amylase Activity activity (Mu)
Rice huller	Malted paddy	41	Dehusked and debranned and grits	127
Centrifugal sheller	Malted paddy	52	Dehusked and broken (brown rice) malt and grits	192
Rubber roll sheller	Malted paddy	68	Dehusked (brown rice) malt and broken	382
Cone polisher	Dehusked (brown rice) malt	56	Debranned malt	256
Horizontal abrasive polisher	Dehusked (brown rice) malt	49	Debranned malt	210
Mini grain mill	Dehusked (brown rice) malt	61	Partially refined malt flour	305
Roller flour mill	Dehusked (brown rice) malt	69	Fully refined malt flour	465

Table 3.4 Yield and composition of milling fractions of malted rice milled in experimental roller flour mill

	B_1	B_2	B_3	C_1	C_2	C_3	BDT[a]	Whole flour[b]	Whole meal	Shorts	Coarse bran
Yield (g%)	5.7	2.6	1.0	33.0	35.5	12.9	1.5	92.2	11.0	5.8	2.0
Moisture (g%)	9.0	9.0	9.0	9.8	9.0	8.8	8.6	9.9	10.0	8.6	8.2
Protein (g%)	3.4	3.9	4.7	5.0	6.0	7.1	8.5	5.2	5.9	9.2	11.6
Ether extractives (g%)	0.5	0.7	1.0	1.2	1.2	1.1	4.2	0.8	1.2	6.1	10.5
Total ash (g%)	0.6	0.9	0.8	0.7	0.7	0.9	3.2	0.9	1.1	4.1	4.5
Acid-insoluble ash (g%)	0.05	0.06	0.07	0.04	0.05	0.04	0.23	0.80	0.12	0.30	0.73
Calcium (mg%)	30.0	28.0	26.0	32.0	26.0	18.0	61.0	9.0	18.0	32.0	41.0
Phosphorus (mg%)	109.0	133.0	124.0	165.0	163.0	238.0	591.0	152.0	188.0	278.0	670.0
Dietary fiber (g%)											
°Soluble	3.2	3.7	2.1	1.2	1.5	1.0	3.0	2.2	–	3.4	3.9
°Insoluble	3.4	3.3	4.5	3.2	3.1	3.3	7.4	4.1	–	23.4	19.4
°Total	6.6	7.0	6.6	4.4	4.6	4.3	10.9	6.3	–	26.8	23.3
Amylase activity (Mu)	548.0	780.0	1,001.0	556.0	371.0	379.0	1,769.0	465.0	395.0	–	–
Viscosity[c] (cP)	740.0	520.0	320.0	500.0	710.0	905.0	1,500.0	1,350.0	2,500.0	–	–
Color (% reflectance)	81.0	79.0	75.0	85.0	75.0	78.0	51.0	75.0	–	–	–

[a] Bran duster through

[b] Obtained by mixing B_1, B_2, B_3, C_1, C_2, C_3, and BDT fractions

[c] At 40% solid content

fractions together amounted to about 75%, but the yield of flour from all the break roll fractions (B_1, B_2, and B_3) together was 10% (Fig. 3.6). The yield of malt flour (straight-run flour) was 92.3% on brown rice basis and 72% on whole rice (paddy) basis. The moisture content of the fractions was about 8%, which showed that the added water was mainly absorbed by the bran layer and did not penetrate into the endosperm. The protein contents of the fractions varied from 3.4 to 8.5%, whereas that of the whole flour was 5.2%. The ether extractive was negligible in all the flour fractions excepting in BDT fraction (4.2%). Total ash content of the flour fractions

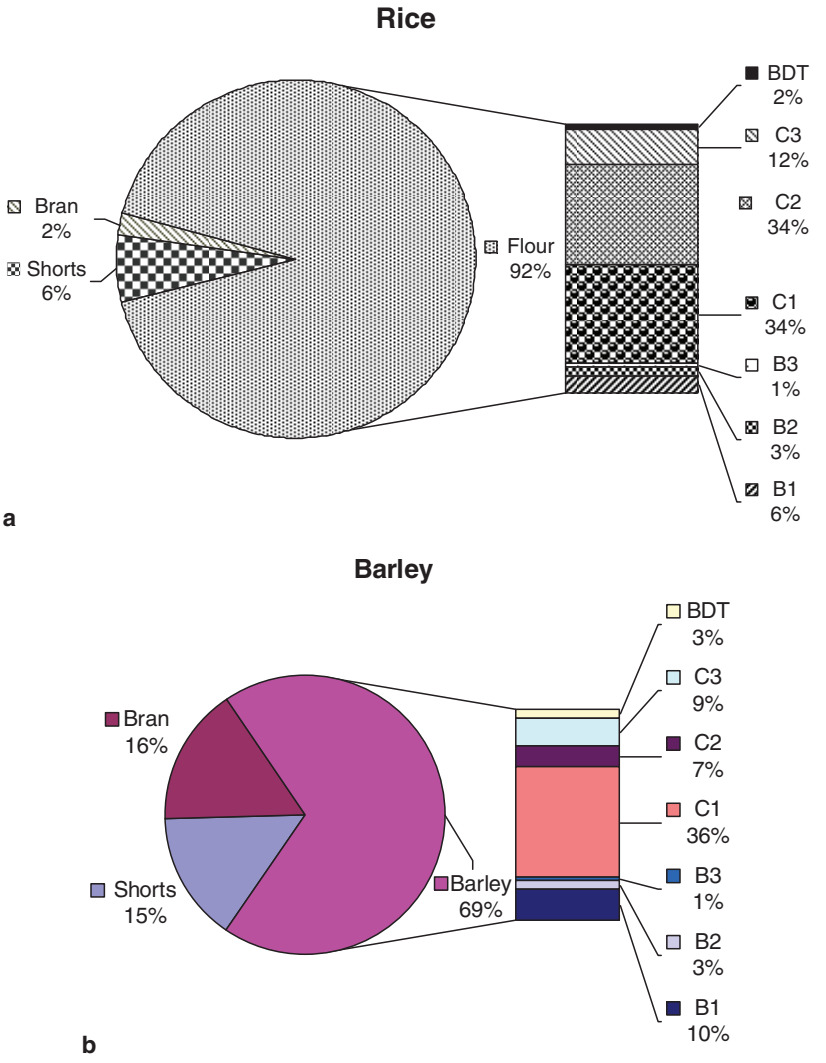


Fig. 3.6 Yield of mill fraction of malted rice and barley, and the individual flour fractions

was also <1% in all the fractions excepting the BDT fraction (3.2%). Similarly, the acid-insoluble ash content was negligible in all flour fractions. Wide variations with regard to amylase activity and viscosity of the flour fractions were observed. Amongst all the mill fractions, the B_3 fraction had the highest amylase activity (1,001 Mu) and the lowest viscosity (320 cP at 40% solid concentration). However, its yield was hardly 1%, and this fraction could be used as a source of amylases. The amylase activity and viscosity of the reduction roll fractions were considerably lower than that of the break roll fractions. Although the yield of BDT fraction was 1.5% of the total flour, it was a good source of protein, fat, minerals, dietary fiber, and amylases (1,769 Mu). However, its viscosity was much higher than other fractions (1,500 cP), which could be due to high levels of its pentosan content. The whole flour contained 5.2% protein, 0.9% ash, and 6.3% dietary fiber, and its viscosity at 40% solids (w/v) was 1,350 cP.

The yield and composition of milled flour fractions of barley malt presented in Table 3.5 indicate that the C_1 fraction is the major flour fraction amounting to 51% of the whole flour (Fig. 3.6). The protein was almost evenly distributed amongst the flour fractions and ranged from 6.4 to 8.3 g%. The ash content was about 1% in all the flour fractions. The B_2 and B_3 fractions contained slightly higher acid-insoluble ash (0.1%) than the other fractions. The amylase activity of the fractions varied from (3,453–4,933 Mu), and the viscosity of the cooked slurry of barley malt was considerably lower than the cooked slurry of rice malt. Interestingly, the viscosity of break roll fractions, namely B_1 and B_2, was considerably lower than the viscosity of reduction roll fractions, although their amylase activity was not very high compared with that of reduction roll fractions. Reduced viscosity in the break roll fractions could be due to high amount of starch content in the B_1 and B_2 fractions coupled with the differences in their dietary fiber contents. Also the high level of soluble dietary fiber (β-glucans) present in the reduction roll fractions might have contributed to higher viscosity in these fractions than in the break roll fractions.

Similar to rice, the BDT fraction of barley was also rich in protein, ether extractives, and dietary fiber contents. The whole flour contained 7.8% protein, 0.8% total ash, and 8.0% dietary fiber. The amylase activity of the whole flour was (3,325 Mu), and its paste viscosity at 40% solids was 440 cP.

The nutritional and textural characteristics of malt flour from rice indicated that it was comparable with other cereal malts in many aspects (Akoma et al., 2006). Rice is known for its low level of fiber content, easily digestible carbohydrates, and good quality protein (Helm and Burks, 1996). Probably, milling of malted rice to obtain edible grade malt flour was one of the major constraints for its widespread malting. This constraint was successfully overcome by adopting innovative method of milling, which involves dehusking the rice malt in rubber roll sheller followed by moist conditioning, grinding, and sieving in roller flour mill (Malleshi et al., 1996; Chakravarthy, 1997). It is worth noting that some of the milling fractions of rice malt are exceptionally rich in energy and have high potential for their use in preparation of cereal-based specialty foods such as infant food, weaning foods, sports foods, and enteral foods, among others (Chakravarty, 1997). Capanzana and

Table 3.5 Yield and composition of milling fractions of malted barley milled in experimental roller flour mill

	B_1	B_2	B_3	C_1	C_2	C_3	BDT[a]	Whole flour[b]	Whole meal	Short	Coarse bran
Yield (g%)	10.4	2.7	1.6	35.0	6.9	8.8	3.5	68.9	100	15.0	16.1
Moisture (g%)	7.7	8.3	8.7	7.4	8.1	8.3	8.9	8.6	8.9	10.5	8.2
Protein (g%)	6.4	7.6	8.2	8.0	8.1	8.3	10.3	7.8	8.6	7.6	9.9
Ether extractives (g%)	0.9	1.3	1.8	1.1	1.4	2.1	3.7	2.1	1.4	1.0	4.3
Total ash (g%)	0.6	0.8	1.0	0.7	0.8	1.2	1.3	0.8	1.8	5.9	5.2
Acid-insoluble ash (g%)	0.08	0.12	0.10	0.03	0.05	0.07	0.30	0.06	0.20	3.06	1.42
Calcium (mg%)	15.3	15.2	24.0	28.4	45.4	38.7	86.8	32.0	41.0	57.8	51.4
Phosphorus (mg%)	135.5	165.1	210.7	203.8	214.0	261.4	286.9	168.0	245.0	221.0	452.0
Dietary fiber (g%)											
Soluble	3.6	4.7	3.8	3.7	4.0	6.5	3.7	3.0	–	–	5.9
Insoluble	3.1	4.4	6.2	4.9	6.2	9.4	10.2	5.0	–	–	50.5
Total	6.7	9.1	10.0	8.6	10.2	15.9	13.9	8.0	–	–	56.4
Amylase activity (Mu)	3,453.0	4,062.0	4,113.0	4,351.0	4,933.0	800.0	3,258.0	3,325.0	3,002.0	420.0	1,670.0
Viscosity[c] (cP)	198.0	196.0	500.0	440.0	900.0	2,700.0	1,400.0	440.0	–	–	–
Color (% reflectance)	70	66	60	68	64		54	51	–	–	–

[a] Bran Duster Through

[b] Obtained by mixing B_1, B_2, B_3,,C_1,, C_2, C_3 and BDT fractions

[c] At 40% solid content

Malleshi (1989) explored the possibility of milling of malted rice in roller flour mill to prepare low-fiber malt flour suitable for weaning foods.

Rice milling is a well-studied subject. It has been documented that rapid drying of paddy containing high moisture causes crack formation (Henderson, 1954; Kunze and Hall, 1965; Choudhary, 1970), which subsequently results in breakage during milling (Rhind, 1962; Velupillai and Pandey, 1990; Afzalinia et al., 2004). While preparing rice malt, the sprouts are dried from about 30% moisture to 10–14% moisture levels within 4–5 h, and the dried green malt is further subjected to kilning (heat treatment). These conditions are conducive to crack formation. Moreover, during malting, the endosperm cells are partially degraded and the rigidity of the starch granules is decreased, thereby softening the endosperm (MacGregor, 1980; Bertoft and Henriksnas, 1983). Therefore, the high breakage that occurs during milling of malted rice is due to the cumulative effect of all these factors. However, the present study has shown that premilling conditioning of the rice malt and careful manipulation of the milling conditions can minimize the breakage of malted rice during milling.

Malted barley has been extensively used for brewing purposes. For brewing, malted barley is milled to coarse grits, and the grits are mixed with water and warmed to facilitate solubilization of the endosperm food reserves. The soluble is extracted and filtered, and the insoluble along with husk or the seed coat is obtained as residues (Hickenbottom, 1996). However, the incipient moist conditioning and milling of barley malt to obtain fiber-regulated, enzyme-rich malt flour carried out in the present study is an innovative method that has shown the potential for preparing barley malt flour almost free from seed coat. The fiber content of the barley malt flour is mostly contributed by β-glucans, which offers many health benefits (Keenan et al., 2007). The dietary fiber of barley exerts hypoglycemic and hypocholesteremic effect besides regulating the bowel movement (Kahlon and Chow, 1997), and therefore, a small portion of bran mixed with the malt flour may be desirable.

Finger Millet Malt

Finger millet contains about 6% protein, and malting further lowers its protein content. Kurien and Doraiswamy (1967) have reported that nearly 30% of its protein is concentrated in seed coat, and therefore, the refined millet malt flour is generally a poor source of protein. However, its bioavailability is much higher than the whole meal. Two-days malted finger millet has been shown to be suitable for specialty foods (Malleshi and Desikachar, 1982). Hence, 2-days malted millet was prepared on pilot scale and milled in a hammer mill to prepare low-fiber malt flour (Malleshi and Desikachar, 1981a). The finger millet malt contributes calcium, methionine, and carbohydrates besides some amount of soluble as well as insoluble dietary fiber to its food (Malleshi and Desikachar, 1986b).

Mung Bean Malt

Mung bean malt is a rich source of protein containing BCAA, carbohydrates, some of the water-soluble vitamins, and the dietary fiber. Many investigators have reported that sprouting mung beam for 24 h is optimum for its utilization in health and enteral foods (Malleshi and Klopfenstein, 1996; Kaur et al., 2007). Accordingly, malt from mung bean was prepared by sprouting the bean for 24 h.

The cooked paste viscosity of native and malted rice, barley, millet, and mung bean presented in Fig. 3.7 indicates that the viscosity of malted cereals is considerably lower than their native counterparts at all comparable solid concentrations.

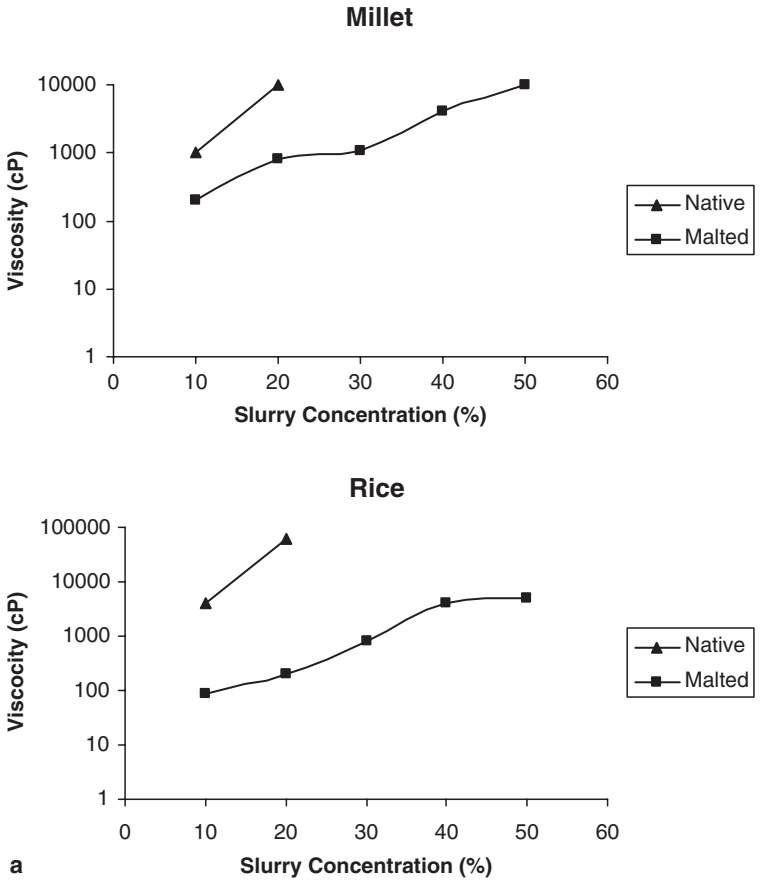


Fig. 3.7 Cooked paste viscosity of native and malted Rice, Barley, Millet and Mung Bean.

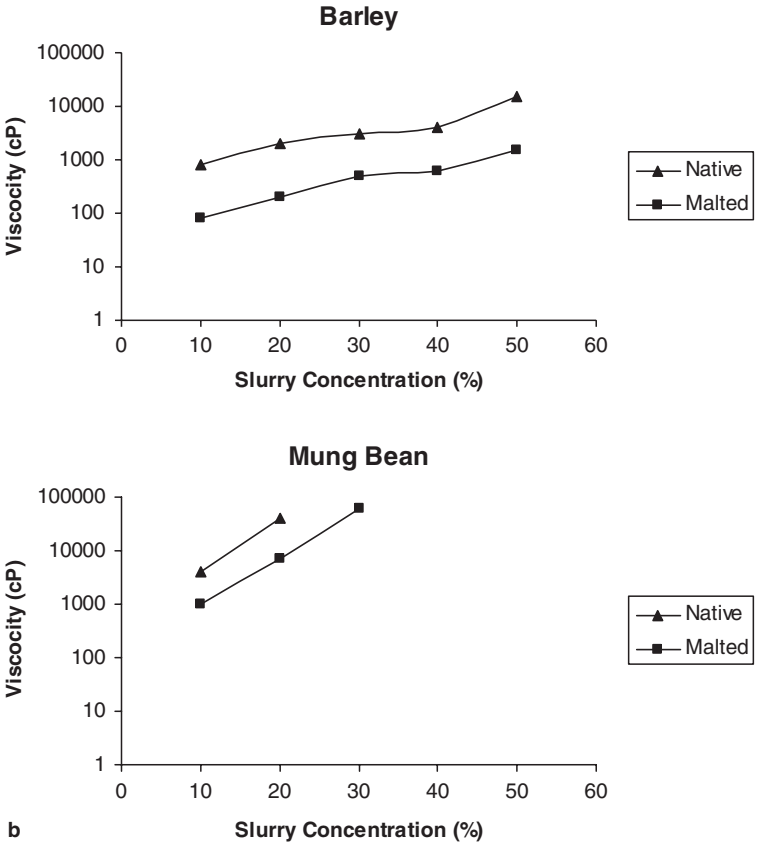


Fig. 3.7 (continued)

However, malting mung beam brought down its viscosity to a smaller extent. Sumathi et al. (1995) have reported that among tropical legumes, although mung bean develops considerable amylase activity during malting, it does not drastically lower the cooked paste viscosity of its malt flour.

Popped Amaranth

Popped amaranth flour is of fluffy and smooth texture with pleasant aroma. Its dispersibility and reconstitution characteristics in water are similar to other popped cereals (Hadimani, 1994). Popped amaranth is known to be a good source of easily digestible carbohydrates, protein, and also good quality dietary fiber (Betschart et al., 1981; Guerra-Matias and Arêas, 2005).

SEM Examination of Cereals and Mung Bean

The SEM photomicrographs of native and malted barley, rice, millet, and mung bean as well as that of native and popped amaranth are presented in Figs. 3.8, 3.9, 3.10, respectively. The native starch granules are of intact nature with protein bodies attached, whereas the starch granules of the malts are almost free from cell walls and protein bodies. Pitting on the granules is prominently visible in case of barley. In rice malt, although there is no visible pitting on the granules, protein matrix covering them has been completely digested by the enzymes. Similarly, in case of millet, the surface erosion is clearly visible and the smaller granules are missing, indicating enzyme action during germination. Similar observations on the electron microscopic examinations of malted cereals have been reported (Selving et al.,

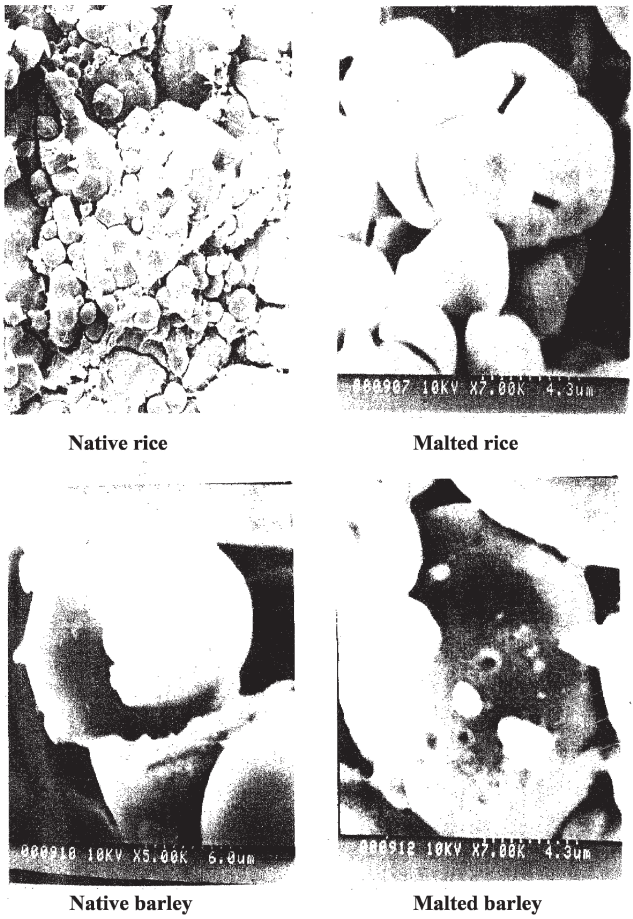


Fig. 3.8 Scanning election photomicrographs of native and malted rice and barley

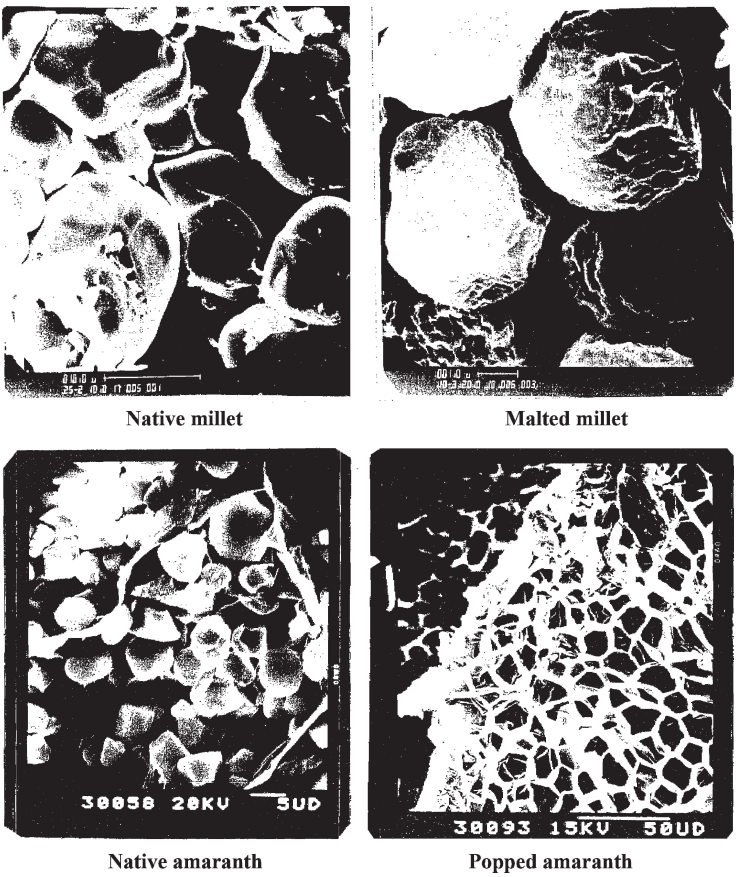


Fig. 3.9 Scanning election photomicrographs of native and malted millet and native and popped amaranth

1986; Brennan et al., 1997; Chakravarty, 1997. On the other hand, not many changes in the structure of starch granule and the cell walls are observed in mung bean malt, which could be due to the short duration of germination (1 day). As expected, the popping has completely disrupted the granular organization and texture of amaranth, and the expanded starch has formed honeycomb structure (Hoseney et al., 1983; Konishi et al., 2004).

Processing of Other Ingredients

Toasted Soya

Toasting the defatted soya reduces moisture level, improves its flavor and the sensory qualities. Heat treatment also helps denature the protein inhibitors and improve

Fig. 3.10 Scanning election photomicrographs of native and malted mung bean

its overall digestibility. Hence, defatted soy flour was given a mild heat treatment prior to its use in the enteral foods.

Milk

For preparation of low-cost general-category food whole milk powder was procured from the local market. For preparation of spray-dried general-category and disease-specific enteral foods, fresh pasteurized milk containing 3.4% protein, 3.25% fat, and 7% nonfat and nonprotein solids was procured from the Karnataka Milk Federation Dairy, India.

Yogurt

The homogenized milk was boiled and cooled to 37°C. To that pure culture of lactic acid Bacillus (Lactobacillus *sporogens*) was added (60×10^8 L^{-1}) and incubated at 37°C. The yogurt thus set was used as natural sources of probiotics to be incorporated in enteral food formulations.

Oil

Edible-grade (BP) fish oil, soy oil (Vital SM, Britannia, Calcutta, India), and coconut oil (Coco care, Mumbai, India) were also procured from local market and incorporated in the enteral foods as sources of lipids.

Hen's Egg

Fresh hen's eggs were purchased from the local poultry farms as and when required.

Gum Arabic/Acacia

Spray-dried edible-grade gum acacia was supplied by Drytech Processes (I) Pvt. Ltd., Mumbai, India.

Vitamin/Mineral and Shadow Nutrients

Both fat and water-soluble vitamins, minerals, and shadow nutrients (taurine and carnitine) of Indian Pharmacopia (IP) or United States Pharmacopia (USP) or British Pharmacopia (BP) grade were obtained from the local pharmaceutical dealers. All the food materials including malt flour were packed in airtight containers and stored in a room maintained at 0°C till use.

Summary and Conclusion

Rice malt was prepared by steeping paddy (rough rice) for 24 h in water, germinating for 72 h, drying the sprouts to about 14% moisture, and kilning the green malt at about 60°C. The malted rice was dehusked in rubber roll sheller and milled in roller flour mill to prepare malt flour. The yield of malt flour was 72% on paddy basis and 92% on brown rice basis. The malt flour contained 5.2% protein and 6.3% dietary fiber; it was a good source of amylases. Barley malt was surface-moistened and milled in roller flour mill to prepare enzyme-rich fiber-regulated malt flour. The yield of malt flour was 69%, and it contained 7.8% protein and 8.0% dietary fiber. Some of the milling fractions of barley malt were exceptionally rich in amylase activity, and some fractions were rich source of dietary fiber. The cooked paste viscosity

of whole malt flour even at 40% solids concentration had free-flowing consistency. Flours from finger millet malt, mung bean malt, and popped amaranth were prepared following standardized methods. The physicochemical characteristics and proximate compositions of malt flour from barley and rice showed that they could be gainfully exploited for the preparation of specialty foods such as enteral foods.

From this study, the following conclusions can be drawn: (1) Rice can be malted similar to other cereals and the physicochemical characteristics of rice malt flour are comparable with that of malt flour from other tropical cereals. (2) To prepare fiber-regulated, enzyme-rich malt flour from rice, the malted rice should be dehusked and the brown rice malt can be milled, whereas to obtain enzyme-rich, fiber-regulated flour from barley malt, it should be moist-conditioned and milled in roller flour mill. (3) By suitably monitoring milling conditions, it is possible to prepare malt flour fractions exceptionally rich in amylase activity.

Chapter 4
Formulation of Enteral Foods

Introduction

This chapter describes the formulation of enteral foods based on malted cereals. Malted cereals are rich sources of easily digestible carbohydrates but are poor sources of dietary proteins. The proteins of malted cereals are deficient in lysine and tryptophan contents. To overcome this deficiency, the malted cereals are generally blended with grain legumes, or protein of animal origin for the preparation of nutritious foods (Chandrasekhara et al., 1957; Deshikachar, 1980; Malleshi and Deshikachar, 1986; Mareo et al., 1988; Onilude et al., 2004; Rao and Muralikrishna, 2006). Grain legumes, milk, and eggs are rich sources of dietary protein and contain favorable profile of essential amino acids (EAA). As discussed earlier, malting or sprouting also improves the overall nutritional quality of cereals and legumes (Bilgiçli and Elgün, 2005). Hence, the blend of malted cereals and legumes will be nutritious foods of enhanced bioavailability of nutrients. Incorporation of milk and egg to the blend further improves the protein quality as well as palatability of the food.

While formulating enteral foods, besides the overall nutritional quality, other factors such as the nutrient density, electrolytes concentrations, content of specific nutrients such as branched chain amino acids (Bower et al., 1986; Ferrando et al., 1995; Kato and Suzuki, 2004), aromatic amino acids (Ferenci, 1996), arginine (Barbul, 1986; Farreras et al., 2005), glutamine (Souba et al., 1990; Gianotti et al., 1995; Van den Berg et al., 2005), nucleotides (Van Buren et al., 1983; Farreras et al., 2005), and shadow nutrients (Menon and Natraj, 1984) among others require considerations, depending on the requirement of patients. Similarly, the composition of lipid, namely the proportion of polyunsaturated fatty acids (PUFA) with special reference to the ratio between n-6 and n-3 fatty acids (Narshimha Rao, 1993; Turley and Strain, 1993; Grester, 1995; Roy et al., 2004; Farreras et al., 2005), structured glycerides (Sandstorm et al., 1995; Roy et al., 2004), and medium-chain triglycerides (Ledeboer et al., 1995) is required to be incorporated in appropriate proportion. The beneficial effects of dietary fiber in normal nutrition are being extended to enteral nutrition (Cummings et al., 1980; Rushdi et al., 2004). The soy polysaccharides (Scheppach et al., 1990; Kapadia et al., 1995; Lien et al.,

M. Kaur, *Medical Foods from Natural Sources,*
DOI: 10.1007/978-0-387-79378-8_4, © Springer Science+Business Media, LLC 2009

1996) are being used as sources of dietary fiber in most of the enteral foods. The breakdown fraction of the dietary fiber of the enteral foods serves as a major fuel to the intestinal villi in the form of short-chain fatty acids (mainly butyrates), besides contributing to the other well-known physiological benefits of the dietary fiber (Heymsfield et al., 1988; Frankenfield and Bayer, 1989; Rays et al., 2005). Several investigators have reported the beneficial effects of probiotics especially of lactic acid bacteria (LAB) in terms of colonization of intestinal microflora, reduction in lactose intolerance, minimization of bacterial translocation, and enhancement of immune status among others (Gilliland and Speck, 1977; Puhan and Zambrini, 1992; Schiffrin et al., 1995; Helander et al., 1997; Patider and Prajapati, 1997; Klaenhammer et al., 2005; Rays et al., 2005; Xiao et al., 2006).

Egg is used as one of the dietary components of patients at hypercatabolic state such as the thermally injured ones (Yu et al., 1988). Often, the raw egg is mashed in milk or other liquids and fed enterally to the patients, because heating coagulates the egg proteins and makes it impossible for tube feeding.

The blend of malted cereals and legumes requires cooking prior to feeding. Hence, to prepare ready-to-use or ready-to-eat enteral foods, the blends need to be processed suitably. Among the various food processing technologies, spray drying is very effective in obtaining a product with minimum loss of nutritional quality (Sharma, 1996; Gardiner et al., 2000; Pisecky, 2005). The spray-dried foods reconstitute in water or other potable liquids easily to a homogenous food suitable for tube or oral feeding. However, preparation of ready-to-eat foods by any of the food processing technologies adds to the cost and to some extent affects the protein quality. Ready-to-eat foods in general and spray-dried foods in particular need special packaging materials also. On the other hand, cereal malt, legume malts, milk powder, and oil may be blended in suiTable proportion, and the same may be fortified with necessary vitamins and minerals to prepare the enteral foods. Although such food needs cooking prior to feeding, it could be cost-effective. Development of such low-cost general-cagory enteral food (GEF-LC) was thought to be useful to provide enteral nutrition support to the patients belonging to developing countries. Hence, a GEF-LC was formulated. In addition, a ready-to-eat general-cagory enteral food (GEF-RT) and a disease-specific enteral food for burn patients (DEF-BP) were also developed. All these three enteral foods were prepared based on natural food ingredients.

The osmolality, renal solute load (RSL), and the nature of ingredients (e.g., intact or elemental) present in enteral foods influence the acceptability, tolerance, and overall effectiveness of enteral nutrition support (Tomarelli, 1976; Martin and Acosta, 1987; Pogacer et al., 2000; Nalin et al., 2004). The enteral foods prepared from natural food ingredients were expected to have favorable osmolality and RSL, and better tolerance than enteral foods prepared from defined ingredients.

The composition of the ingredients, the processing methodology followed, the nutrient contents, physicochemical characteristics, and microbiological profiles of the enteral food formulations are discussed in detail in this chapter.

Materials and Methods

Formulation of General-Category Enternal Foods

Low-Cost Food

Malted rice, malted barley, malted finger millet, malted mung bean, defatted and toasted soy flour, whole milk powder, soy oil, coconut oil, and gum acacia were blended in a Hobart Mixer, and the blend was fortified with vitamins, minerals, shadow nutrients, and conditionally essential nutrients. Dried form of lactic acid bacillus (15×10^7 counts per 100 g) was added as a source of LAB. This food was termed as GEF-LC. The proportion of ingredients of the enteral food is given in Table 4.1, whereas the amounts of micronutrients added are given in Table 4.2.

Ready-to-Eat Food

Rice malt, barley malt, millet malt, mung bean malt, toasted soy flour, soy oil, coconut oil, and gum acacia were mixed in potable water and to that, fresh pasteurized milk was added (Table 4.1). The contents were heated in a steam-jacketed cooker gradually increasing the temperature with constant stirring till boiling. The cooked mass was cooled to room temperature (25°C), and yogurt containing active bacterial culture namely LAB was added to it (Table 4.2). The contents were homogenized in a colloidal mill and spray dried in a pilot-scale spray drier (drying capacity 30 L h^{-1}, rpm of atomizer 30,000, inlet air and drying chamber temperatures

Table 4.1 Ingredients and proportions of enteral foods (per 100 g)

Ingredients	GEF-LC and RT	DEF-BP
Malted barley	22.0	21.0
Malted rice	10.0	5.0
Malted finger millet	13.5	6.0
Malted mung bean	20.0	5.0
Defatted soya	5.0	5.0
Popped amaranth	–	10.0
Egg (dwb)	–	18.0
Milk (dwb)	18.0	18.0
Yoghurt (dwb)	2.0	2.0
Soy oil	6.5	2.0
Fish oil	–	5.0
Coconut oil	1.0	1.0
Glycerine	1.0	1.0
Gum acacia	1.0	1.0

Table 4.2 Added micronutrients to the enteral foods (per 100 g)

Nutrients	GEF-LC and RT	DEF-BP
Vitamins		
Vitamin A (acetate) (IU)	500.0	2,000.0
Cholecalciferol (IU)	50.0	200.0
α-Tocopherol acetate (mg)	17.9	17.9
Vitamin K (μg)	5.0	5.0
Thiamine (μg)	0.5	0.5
Riboflavin (mg)	0.5	0.5
Pyridoxine hydrochloride (mg)	0.2	0.2
Cyanocobalamine (μg)	0.8	0.8
Nicotinamide (mg)	5.0	5.0
Calcium pantathonate (mg)	0.8	0.8
Ascorbic acid (mg)	20.0	30.0
Biotin (mg)	0.01	0.01
Choline (mg)	100.0	100.0
Minerals		
Calcium (mg)	10.0	10.0
Phosphorus (mg)	19.0	19.0
Iron (mg)	10.0	10.0
Iodine (μg)	28.5	28.5
Zinc (mg)	10.5	15.0
Copper (mg)	0.2	0.2
Magnesium (mg)	6.6	10.0
Chromium (μg)	2.0	3.6
Selenium (μg)	4.6	4.6
Molybdenum (μg)	7.7	7.7
Sodium (mg)	–	50.0
Shadow nutrients		
Taurine (mg)	16.0	16.0
Carnitine (mg)	12.0	12.0
l-Glutamine (mg)	125.0	250.0
Active bacterial culture		
LAB (cfu g^{-1})	5.4×10^6	5.4×10^6

170 and 90°C, respectively) to prepare GEF-RT. The enteral food thus prepared was aseptically collected and was fortified with vitamins, minerals, and other conditionally essential and shadow nutrients.

Formulation of Disease-Specific Enteral Foods

Enteral Foods for Burn and Hypercatabolic Patients

Generally eggs form an important component for patients at hypercatabolic state such as burns or major surgery. Hence, it was felt appropriate to incorporate eggs as one of the constituents of the enteral food for burn patients. Accordingly, malt

flour from rice, barley, finger millet, and mung bean, milk, and eggs were mixed, and the slurry was cooked under controlled temperature and pressure to facilitate the enzyme activity of malt flour. But while cooking, the egg protein coagulated rendering it unsuiTable for spray drying. To overcome this difficulty, attempts were made to hydrolyze the egg protein using proteolytic enzymes such as pepsin, trypsin, and chymotrypsin. However, these attempts did not yield satisfactory result under feasible conditions for preparing the enteral food. After several laboratory trials, this difficulty was overcome by following suiTable mixing sequences of ingredients (Malleshi and Chakraverty, 1996). Accordingly, cereal malt, mung bean malt, toasted soy flour, popped amaranth, egg, soy oil, fish oil, gum acacia, and fresh milk were blended (Table 4.1). The blend was fortified with active bacterial culture (Table 4.2) and processed similar to that followed for the preparations of the GEF-RT. This food was named as DEF-BP.

After successfully preparing the GEF-RT and DEF-BP in pilot scale (batch of 10 kg), large-scale (200 kg) batches of both the foods were prepared in an industrial scale spray drier. GEF-LC, GEF-RT, and DEF-BP were packed in moisture-proof paper-poly-foil-laminate pouches immediately after preparation and stored in a room maintained at 0 C. The foods were used for determining physicochemical characteristics, protein quality evaluation, and shelf-life studies and for conducting clinical trials in various hospitals.

A proprietary enteral food based on defined ingredients (maltodextrin, hydrolyzed protein, soy oil, and sugar) procured from the local market was used for the comparison of the physicochemical characteristics and the protein quality evaluation with the enteral food formulations from natural sources.

Physicochemical Characteristics

Physical Features

The visible observations such as color, particle size, and hand feel of the dry foods were recorded.

Bulk Density

The apparent volume of a known weight of the foods was measured in a measuring cylinder, and the bulk density was calculated as weight (g)/volume (mL).

Reconstitution Characteristics

Fifty grams of the foods were dispersed in 150-mL warm water (GEF-LC was cooked), mixed well, and the slurry was transferred to a 250-mL measuring cylinder.

After allowing the reconstituted foods to stand for an hour, homogeneity and phase separation characteristics of the foods were observed at hourly interval up to 6 h.

Viscosity

The GEF-LC (15–40% w/v) was dispersed in cold water and the slurry was heated slowly on water bath to boiling. The cooked slurry was cooled to room temperature, and the viscosity was measured in Brookfield viscometer using appropriate spindles depending on the slurry consistency.

The GEF-RT and DEF-BP (15–40% w/v) were dispersed in warm water (37°C), mixed well, and the viscosity was determined following the same procedure as that of GEF-LC.

Osmolality

The GEF-LC was cooked in water, whereas the GEF-RT and DEF-BP were reconstituted in warm water and their osmolalities were measured in a osmometer (Osmomat–030, Gonoec gmbH, Germany).

Renal Solute Load (RSL)

The RSL was calculated as per the formula suggested by Ziegler and Fomon (1971).

RSL (mOsmol L^{-1}) = Sum of mEq of Na, K, and Cl plus 4 mOsmol urea per g of protein in a liter of the food.

Flow Behavior

Two-hundred milliliters of reconstituted enteral foods (20–35% solid concentration) taken in delivery funnels fitted with 6–18-FG Ryle's tubes was allowed to flow from a height of about 3 ft from the working Table under gravity. The flow time was recorded up to the fraction of a minute. In a parallel experiment, the foods at 10–30% concentration with 5% increment were evaluated for their flow characteristics through 12-FG Ryle's tube.

Solubility Index and Swelling Power

The solubility indices and swelling power of the enteral foods in an aqueous system were determined according to the method of Anderson et al. (1969). One gram of

sample was dispersed in 15-mL distilled water and heated for 30 min in a shaking water bath at predetermined temperature from 25 to 85°C. The contents were centrifuged at 5,000 rpm for 20 min. The supernatant was decanted and dried at 105°C and weighed. The water solubility index (WSI) was calculated as percentage of dry solids. The water adhering to the side of the tube was wiped off and the residue was weighed. Swelling power or water absorption index was calculated based on weight (g) of gel per gram of dry sample.

Proximate Composition

The formulated enteral foods were analyzed for moisture, crude protein (micro-kjeldahl), total ash and acid-insoluble ash, and calcium and phosphorus contents as per the approved method of AACC (1983). Total lipid was estimated following the method of Roese-Gottleib as per AOAC (1980). Total, soluble, and insoluble dietary fiber components of the foods were determined according to the method of Asp et al. (1983).

Carbohydrate Profile

Free Sugars

Defatted samples were refluxed with 70% ethanol, and the extract was purified by passing through cation (Doex 1) and anion (Doex 50) resin, and concentrated under low temperature in a rotary flash evaporator. The concentrated sugar solution was filtered through millipore filter, and the total sugar content of the solution was estimated by phenol-sulfuric acid method (Dubois et al., 1956). The component sugar of ethanol extract was detected by paper chromatography.

Gel-Permeation Chromatography of Carbohydrates

The carbohydrate profiles of the foods were determined by gel permeation chromatographic separation technique as per the method of Chinnuswamy and Bhattacharya (1986). About 50 mg of defatted sample was taken in a centrifuge tube, 4 mL of 90% DMSO was added and boiled in water bath for 15 min and incubated at 70°C for 1 h (GEF-LC was cooked in boiling water bath, freeze dried, defatted, and used for fractionation). The volume was made up to 15 mL and centrifuged at 1,000 rpm for 20 min. The total sugar content of supernatant was estimated by phenol-sulfuric acid method. An aliquot of the supernatant corresponding to 10-mg carbohydrates (dwb) was fractionated by ascending chromatography on a Sepharose CL-2B (Pharmacia Fine Chemicals, Sweden) column (1.6 cm × 70 cm), operating with peristaltic pump at a flow rate of 15 mL per hour using double distilled water

containing 0.02% sodium azide as an eluent. Total eluted volume of 150 mL was collected into 3-mL fractions. An aliquot of 0.5 mL was used for determination of carbohydrate content using phenol-sulfuric acid method measuring the absorption at 490 nm.

The void volume and the total volume of the gel were determined using blue dextrans and glucose, respectively. The approximate molecular weights were determined from a standard curve prepared by running different dextran standards (Pharmacia Fine Chemicals, Sweden). A blend of unmalted cereals, legume, milk, egg, and soy taken in a proportion similar to that used for enteral food formulations was mixed with water; the slurry was cooked, homogenized, and also spray dried. The carbohydrate profiles of these control foods (native cereal counterparts of the enteral foods) were also determined similarly.

Protein Profile

Amino Acids

Samples equal to 10 mg of protein were weighed into 16 mm × 25 mm culture tubes to which 6N HCl under vacuum was added. The tubes were capped and placed in a closed water bath set at 110°C for 24-h hydrolysis. Upon completion of hydrolysis, samples were dried and resuspended in 50 mg per milliliter potassium EDTA solution. A 1:100 dilution of the suspension was made, and 10 mL of this dilution was analyzed for amino acid composition on an Applied Biosystem (Model: 420 A) amino acid analyzing system (Jones, 1981).

Protein Fractionation by SDS-PAGE

Twenty milligram of each of the defatted food samples was dissolved in 300 mL of 4% sodium dodecyl sulfate (SDS)/2 M urea/5%, 2-mercaptoethanol (ME), vortexed and centrifuged for 10 min at 10,000 rpm. The extracted supernatants were mixed with an equal volume of SDS sample buffer (0.125 M Tris-HCl of pH 6.8, SDS 4% w/v, 2 M urea, 2-ME 5% w/v) and heated at 96°C for 3 min in a water bath.

The protein extract (20 μL) was subjected to fractionation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) at a constant current of 30 mA on a vertical electrophoreses system until the dye front reached the other side of the gel. The gel was stained for protein using coomassie brilliant blue R-250 (0.05%) in a methanol–acetic acid–water (25:10:65) solvent system and destained in the above solution without the dye.

The protein molecular markers used were lysozyme (14,300), carbonic anhydrase (29,000), ovalbumin (43,000), bovine serum albumin (68,000), and phosphorylase b (97,400). The relative molecular weights were plotted against the distance of the protein migrated, and the molecular weight of the proteins in the food samples was determined with reference to this curve.

Lipid Profile

Total Lipid

Four grams of the sample was suspended in 10 mL of water in a Mojonnier fat extraction tube. To that 1.2 mL of NH_4OH was added followed by 10 mL of alcohol and mixed well. To the contents, 25 mL of diethyl ether was added, and the contents were shaken vigorously for 30 s. Finally, 25 mL of petroleum ether (40–60°C) was added and shaken again for 30 s, and allowed to rest for 20 min or till the separation of liquid phase was complete. Ether-fat layer was drawn off carefully to a preweighed flask. Extraction was repeated two more times using 15 volumes each of diethyl ether and petroleum ether (previously mixed and free from deposited water). The solvents were made to evaporate by warming the extract on a steam bath and then the fat was dried at 100°C in an air oven to constant weight as per the method of Roese-Gotteleib (AOAC, 1980).

The lipids of the food samples were extracted as described earlier, and the lipid extracts were flushed with nitrogen gas and used for the estimation of cholesterol, phospholipids, and fatty acid contents.

Cholesterol

The lipid extract was saponified with 0.3 mL of 33% ethanolic KOH and incubated at 65°C for 15 min, cooled, and to that 10-mL hexane and 5-mL water were added, mixed well, and the known amount of hexane extract was taken for cholesterol estimation by $FeCl_3$ method (Zlattis and Zak, 1969). The Stock $FeCl_3$ was prepared by dissolving 504-mg anhydrous $FeCl_3$ in 10-mL acetic acid. The hexane layer was evaporated, and to that, 1.5 mL of working standard (1:100 dilution of stock $FeCl_3$) was added and allowed to stand for 10 min. Then, 1 mL of concentrated H_2SO_4 was added, mixed, and the contents were placed in dark for 45 min, and the absorbance was measured at 560 nm.

Phospholipids

Phospholipids were quantified by ferrous ammonium thiocyanate method, using dipalmitoyl phosphatidyl choline (10–100 g) as reference standard. The known amount of lipid extract was dissolved in 2 mL of chloroform. Two milliliters of ammonium ferrothiocyanate was added and mixed for 1 min and left for 10 min and then centrifuged. Following the phase separation, absorbance of chloroform phase was measured at 488 nm (Stewart, 1980).

Fatty Acids

The lipid extracts were saponified with 1 mL of 0.5 M KOH in methanol by keeping the tubes in a water bath for 10 min at 86°C. Unsaponified material was removed

using 2-mL hexane. The solution, after removal of unsaponified materials, was acidified with 1 mL of 0.7 methanolic HCl. Fatty acids were extracted from the acidified solution with hexane (2 mL × three times). The pooled hexane layers were evaporated at 40°C under nitrogen. Fatty acids were methylated by adding 0.2 mL of benzene + 0.5 M of 14% BF_3/methanol and by keeping in a water bath at 86°C for 10 min. The fatty acid methyl esters were extracted with hexane (2 mL × three times). Pooled hexane fractions were washed with 5 mL of water, and hexane layers were evaporated by passing nitrogen gas, and then redissolved in benzene (Morison and Smith, 1964).

The methyl esters of fatty acids were separated using fused silica capillary column 25 m × 0.25 mm (i.d. Parmabond FFAP-DF-0.25, Germany) using Shimadzu gas chromatograph (model 14B) fitted with a flame ionization detector. The column temperature was programmed from initial temperature of 160 to 240°C at a rate of 6°C min^{-1}. The injector and detector temperatures were 210 and 250°C, respectively. Nitrogen gas was used as carrier gas (45 mL min^{-1}). Individual fatty acids were identified by comparing the retention times of authentic standards. Fatty acid peaks were integrated, and the areas were quantified using Shimadzu CR4A model integrator.

Microbiological Profile

The microbiological safety evaluation of the foods was carried out according to the methodology suggested by Vanderzant and Splittstoesser (1992).

Total Plate Count and Differential Counts

Appropriate dilutions of enteral foods were prepared in sterile peptone water and were pour-plated using PCA medium in duplicate for total plate counts. Simultaneously, the diluted samples were pour-plated using MRS, KF, streptococcal agar, EMB agar, MCA, PDA, BCA, and BPA media for lactics, fecal streptococcal, E. coli, coliforms, yeast and mold, B. cereus and S. aureus counts, respectively. In both the cases, the plates were incubated at 37°C for 24–48 h, and the total colony was counted and expressed as $\log_{10}$ cfu g^{-1}.

Spore Formers Count

Appropriate dilutions of the products were prepared and treated at 85°C for 30 min for mesophilic, and 100°C for 30 min for thermophilic spore formers. The treated samples were pour-plated using BCP medium and incubated at 37 and 55°C for 24–45 h for mesophilic and thermophilic spore formers, respectively. The colonies were counted and expressed as $\log_{10}$ cfu g^{-1}.

MPN Technique for Coliform Count

The three-tube MPN technique was followed for the detection of coliforms. Appropriately diluted samples (0.1, 0.001, and 0.0001) were added to sterile tubes (three tubes/dilution) containing Mac Conkey broth with Durham's tubes. The tubes were incubated at 37°C for 48 h and observed for growth of coliforms, acid and gas production. The results were computed using three-tube MPN table.

Confirmation of *E. Coli*

One loopful of culture growth from individual positive tubes was surface streaked on EMB plates and also inoculated onto tube containing EC broth with MUG. The EMB plates were incubated at 37°C for 48 h. The colonies appearing as dark green with metallic sheen were scored as *E. coli*. The EC broth tubes with inoculum were incubated at 44°C for 24 h. Acid and gas production with blue fluorescence under long-range UV was confirmed as *E. coli*.

Salmonella Count

Twenty-five grams of the samples was taken in 225 mL of the diluent. An aliquot (10 mL) was enriched in 10-mL lactose broth (DS), incubated for overnight, and then transferred to TTB and SCB tubes for further enrichment for 24 h. The enriched broth of TTB and SCB tubes was streaked on SSA, BSA, and BGA plates and incubated for 48 h at 37°C. Observations for typical Salmonella colonies were recorded.

Pesticide Residue Analysis

The organochlorine insecticide residues in the enteral foods were determined by AOAC (1980) method. To the 20-g sample, 100 mL of 35% water–acetonitrile mixture was added and stirred intermittently for 1 h, and filtered through Whatman No. 1 filter paper. The filtrate was collected in a separate conical flask. The residue was transferred back into the beaker. The process was repeated for two more times and the filtrate was pooled. The acetonitrile extract was transferred into a 2-L separating funnel and diluted with 600-mL water. Ten milliliters of saturated sodium chloride solution was added and shaken gently. The pesticide residue was extracted from the diluted acetonitrile with 100-mL petroleum ether for three times. All the extracts were pooled and concentrated to 5 mL using vacuum evaporator. This was preserved for florosil column cleanup.

Florosil Cleanup

A 22-mm i.d. and 30-cm-long glass column with a stopcock below were used. The lower opening was plugged with glass wool, and the column was filled with activated florosil up to 10-cm height. This was topped with anhydrous sodium sulfate up to 1-cm height. The sample extract was transferred into the column. The column was eluted with 200 mL each of 6, 15, and 50% diethyl ether in petroleum ether at the rate of 5 mL min^{-1}. All the eluted materials were pooled and concentrated in a vacuum evaporator to 2 mL. This concentrate was used for gas liquid chromatography (GLC) to identify the pesticide residues.

The cleaned up extract (1 mL) was injected into GLC (Shimadzu 15A GLC with Ni 63 detector and microprocessor attachment). The column was filled with 1.5% OV-17 on chromosorb W. The column, injector, and detector temperatures were maintained at 220, 225, and 230°C, respectively. Nitrogen flow was 40 mL min^{-1}. The pesticide residue analyses were carried out using the common pesticides standard (Table 4.3).

Calculations

$$\text{Pesticides (PPM)} = (\text{Sample area/Std area}) \times (\text{Conc. of Std./Sample injected}) \times (\text{Volume made up/sample weight in grams}) \times 10^{-6}$$

Results and Discussion

Results

The nutrient composition and EAA contents of the ingredients used for the enteral foods are presented in Tables 4.4 and 4.5. The protein contents of the malted rice (5.2%) and millet (4.2%) were slightly lower, whereas the protein contents of popped amaranth flour were relatively higher (14%) than barley malt (7.8%). As expected, the cereal malt and the popped amaranth were rich sources of carbohydrates. Mung bean malt contained 22% protein whereas defatted soy contained 50% protein. Milk and eggs were also rich sources of protein, fat, and minerals. The dietary fiber content of the enteral food is mainly contributed by barley, mung bean, and soy flour. Finger millet, soy flour, and milk are rich in calcium and phosphorus also. The PUFA of the foods are mainly derived from soy oil and fish oil whereas the medium-chain fatty acids are from coconut oil.

Table 4.3 Common pesticide standard

Pesticide standard	Amount injected (pg)	Area retention	Time (min)
α-BHC	203	85,882	2.4
γ-BHC	270	80,683	2.9
β-BHC	216	24,464	3.2
δ-BHC	180	54,254	3.8
PP-DDT	400	31,553	11.7
PP-DDE	1,560	368,109	8.1

Table 4.4 Nutrient composition of enteral food ingredients

	Rice[a] malt	Barley[b] malt	Finger[b] millet malt	Popped[c] amaranth seed
Protein (g%)	5.2	7.8	4.2	14.0
Fat (g%)	0.8	2.1	1.1	1.0
Carbohydrate (g%)	86.8	80.8	93.0	83.5
Minerals (g%)	0.9	0.8	1.7	1.5
Dietary fiber (g%)	6.3	8.0	7.0	12.1
Calcium (mg%)	9.0	22.0	302.0	510.0
Phosphorus (mg%)	152.0	168.0	157.0	397.0
EAA (gram per 100-g protein)				
Hisdine	–	2.4	3.2	2.3
Isoleucine	–	3.6	3.1	3.6
Leucine	–	7.4	9.1	5.2
Lysine	–	3.6	3.1	4.4
Methionine	–	1.4	3.3	1.9
Cystine	–	2.3	1.9	1.7
Phenylalanine	–	5.9	6.0	3.4
Tyrosine	–	3.6	4.4	2.7
Threonine	–	3.3	4.3	3.5
Tryptophan	–	1.6	1.6	0.9
Valine	–	5.3	5.4	4.3

[a]Analysed values
[b]Malleshi (1992)
[c]Betschart et al. (1981)

Table 4.5 Nutrient composition of enteral food ingredients

	Mung[b] bean malt	Defatted[c] soy flour	Milk[c]	Egg[c]
Protein (g%)	22.0	50.0	26.7	50.0
Fat (g%)	1.5	1.1	27.6	50.0
Carbohydrate (g%)	73.3	42.0	39.5	–
Minerals (g%)	2.8	7.0	6.2	3.0
Dietary fiber (g%)	7.1	3.5	–	–
Calcium (mg%)	75.0	240.0	984.0	228.0
Phosphorus (mg%)	405.0	690.0	756.0	830.0
EAA (gram per 100-g protein)				
Hisdine	3.5	2.4	2.7	2.4
Isoleucine	3.2	5.1	5.4	6.6
Leucine	9.0	7.7	9.6	8.3
Lysine	6.8	6.4	8.0	7.0
Methionine	1.2	1.3	2.3	3.4
Cystine	0.5	0.8	0.8	1.1
Phenylalanine	6.2	4.8	5.1	5.8
Tyrosine	3.3	3.4	4.8	4.0
Threonine	3.7	3.8	4.5	5.1
Tryptophan	1.0	1.3	1.4	1.4
Valine	3.9	5.1	6.4	7.2

[a]Malleshi (1992)
[b]Gopalan et al. (1989)

The compositions of the ingredients of both the general-cagory and disease-specific enteral foods were adjusted in such a way that the foods not only meet the requirement of the macro and micronutrients, but also take care of the conditionally essential nutrients. The malted cereals formed the major ingredients (nearly 45% of the GEF-LC and GEF-RT and 32% of the DEF-BP), enteral foods (Table 4.1). The mung bean content was 20% in case of GEF-LC and GEF-RT but was only 5% in case of DEF-BP. Besides protein, mung bean also serves as a source of branched chain amino acids (BCAA). Milk was one of the major ingredients in both the foods. Soy flour serves as a major source of polysaccharides (dietary fiber) besides contributing to the protein content of the foods (Kapadia et al., 1995). Popped amaranth (10%) and eggs (18%) were the additional ingredients of DEF-BP only. Conventionally, eggs are given to the burn patients (Wagner et al., 1988) because of their protein of high biological value containing albumin. Soy oil and coconut oils were added to both the foods whereas fish oil was added only to DEF-BP as a source of n-3 poly unsaturated fatty acids, which helps improve the immune status in burn patients (Gennari et al., 1995; Gerster, 1995; Kreymann et al., 2006). Yoghurt, glycerine, and gum acacia were also the ingredients for both the foods. Vitamin A, vitamin D, vitamin C, magnesium, and glutamine contents were slightly higher in DEF-BP than in GEF-LC and GEF-RT (Table 4.2).

The foods were of creamy white color, smooth texture and had desirable taste and aroma. However, the DEF-BP had perceptible egg taste. The bulk density of GEF-LC, GEF-RT, and DEF-BP was 0.625, 0.841, and 0.725 g mL^{-1}, respectively.

The proximate composition and the EAA content of the enteral foods are presented in Table 4.6. The proteins and fat contents of general-cagory foods were 16 and 12% (at 25% solid content), and of disease-specific food were 21% and 19% (at 30% solid content), respectively. The GEF-LC, GEF-RT, and DEF-BP contained 4.5, 4.8, and 5.0% dietary fiber. The foods had favorable amino acid composition with their lysine content ranging from 4.7 to 6.0%. The carbohydrate, protein, and fat calories of the general-cagory and disease-specific enteral foods were about 58, 16, 26%, and 44, 19, and 37%, respectively (Fig. 4.1).

The food reconstituted easily in water and also in other potable liquids such as milk and fruit juice. As indicated earlier, the GEF-LC requires cooking prior to feeding. During cooking, the enzymes hydrolyze the starch, reduce the water holding capacity, and increase calorie density of the foods (Malleshi and Desikachar, 1986a). The reconstituted foods were of uniform texture and consistency without settling of particles or phase separation even after 5 h of preparation.

The calorie, protein, and other nutritional information of reconstituted enteral foods in water are presented in Table 4.7. This table reveals that a litter of reconstituted GEF-RT and GRF-LC provides about 1,100 kcal and 40-g protein (at 25% solid concentration) whereas a litter of DEF-BP provides about 1,600 kcal and 63-g protein (at 30% solid concentration). However, the foods can be delivered up to 35% solid content through feeding tubes if high concentrations of nutrients are required. In addition, preparation of foods in milk and addition of sugar to the foods further enhance the protein and energy density of the foods.

Table 4.6 Proximate composition and EAA content of enteral foods

	GEF-LC	GEF-RT	DEF-BP
Moisture (g%)	6.3	2.9	2.0
Protein (g%)	16.0	16.0	21.0
Fat (g%)	12.0	12.0	19.0
Available carbohydrate (g%)	59.2	61.9	50.5
Energy (kcal)	409.0	419.0	457.0
Minerals (g%)	2.0	2.6	2.5
Total dietary fiber (g%)	4.5	4.8	5.0
Soluble dietary fiber (g%)	2.5	2.7	2.7
Insoluble dietary fiber (g%)	2.0	2.1	2.3
Calcium (mg%)	265.0	265.0	300.0
Phosphorus (mg%)	264.0	264.0	314.0
EAA (gram per 100-g protein)			
Hisdine	3.1	2.9	2.5
Isoleucine	2.9	2.4	2.7
Leucine	6.2	6.1	5.9
Lysine	6.1	4.7	5.0
Methionine	2.7	2.4	2.6
Cystine	0.5	0.7	0.5
Phenylalanine	6.4	7.2	7.0
Tyrosine	0.5	2.2	2.1
Threonine	4.2	4.0	4.2
Tryptophan	1.2	1.1	1.2
Valine	6.0	3.4	4.7

The solubility and swelling patterns of foods at different temperatures are shown in Fig. 4.2a, b. The solubility of GEF-LC was lower than that of GEF-RT and DEF-BP up to 60°C. Beyond this, it increased gradually up to 85°C, whereas the solubility of GEF-RT and DEF-BP was around 50% at 30°C and increased slightly as temperature increased. The pattern of solubility and swelling indicated that the GEF-LC contained ungelatinized starch and required cooking whereas the GEF-RT and DEF-BP were precooked and were ready-to-eat foods.

The viscosities of the ingredients and the enteral foods at different solid concentrations are shown in Fig. 4.3a, b. The viscosity of malted barley, rice, and millet was several fold lower than their native counterparts at all comparable slurry concentrations. Malted mung bean was much viscous as compared with malted cereals. Popped amaranth, egg, and soy flour exhibited very high viscosity and formed dough-like texture at 20% solid concentration. However, the viscosity of the enteral foods was lower than cereal malts at all comparable solid concentrations. The foods, even at 35% solid concentrations, were of free-flowing consistency (Fig. 4.3b).

The flow characteristics of the enteral foods at 10–35% solid concentration through 6–18-FG bore size feeding tubes presented in Fig. 4.4a–d indicate that as the concentration of the food increased, the flow time also increased, and that larger bore feeding tubes were needed for a smooth flow. The DEF-BP required longer time than GEF-LC and GEF-RT under similar conditions of concentration and bore

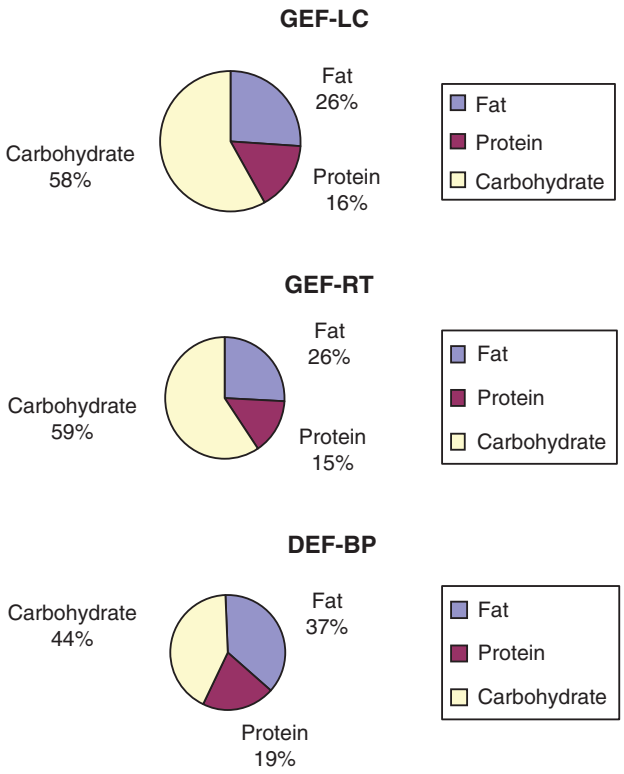


Fig. 4.1 Calorie distribution of enteral foods

size. The flow behavior of GEF-LC and GEF-RT was similar. The foods at 20% slurry concentration passed through 6-FG bore tube without any obstruction. The flow time required by the proprietary food (PEF) was much lower than the developed formulations under all comparative food concentrations as well as feeding tubes (Fig. 4.4d). In practice, however, predetermined flow rate is maintained with the help of pump attached to the feeding accessories.

The osmolalities and the RSLs of the foods at different slurry concentrations are presented in Fig. 4.5a, b. Even at 30% solid content, the osmolality of the foods was within the acceptable limit. The osmolality of the enteral foods at 25% slurry concentration lies in the range of suggested osmotic load (300–650 mOsmol kg^{-1}, Fig. 4.5a) of enteral foods (Zarling et al., 1986)

Likewise the RSLs of the GEF-LC, GEF-RT, and DEF-BP at 25% solid content were 195, 197, and 287 mOsmol L^{-1}, respectively, and were also within the desirable range of 300–900 mOsmol L^{-1} (Tomarelli, 1976). The osmolalities of the malt-based

Table 4.7 Nutritional information on reconstituted enteral foods

Form	GEF-LC[a]	GEF-RT[a]	DEF-BP[b]
Kcal L^{-1}	1,100.0	1,100.0	1,600.0
Protein (% calories)	15.6	15.0	18.3
Carbohydrate (% calories)	57.9	59.1	44.2
Fat (% calories)	26.4	25.7	37.4
Protein (g L^{-1})	40.0	39.5	63.0
Carbohydrate (g L^{-1})	145.0	147.0	152.0
Fat (g L^{-1})	30.0	30.0	57.0
Total dietary fiber(g L^{-1})	11.2	12.0	15.0
Cal:N	160.0	165.0	136.0
Sodium (mg L^{-1})	215.0	215.0	330.0
Potassium (mg L^{-1})	1,012.0	1,012.0	630.0
Osmolality (mOsmol per kg water)	246.0	400.0	500.0
Renal solute load (mOsmol L^{-1})	195.0	197.0	219.0
Volume to meet % (mL) of RDA for vitamins and minerals	1,600.0	1,600.0	1,600.0
Protein sources	Legume, soy Milk	Legume, soy milk	Egg, milk, legume, soy
Carbohydrate	Cereal, milk	Cereal, milk	Cereal, milk
Sources	Legume	Legume	Legume
Lipid sources	Soy oil, milk, coconut oil	Soy oil, milk, coconut oil	Soy oil, milk, coconut oil, fish oil
Intended use	General-category patients	General-category patients	Burn, trauma, sepsis, AIDS, hypercatabolic patients

[a]At 25% solids contents
[b]At 30% solids contents

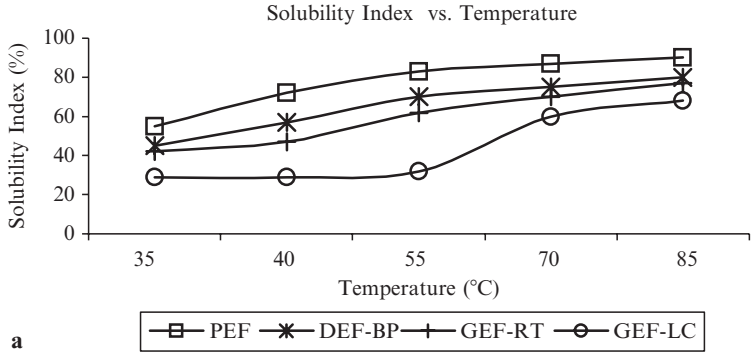


Fig. 4.2 **(a)** Solubility index of enteral foods, (**b**) Swelling power of enteral foods

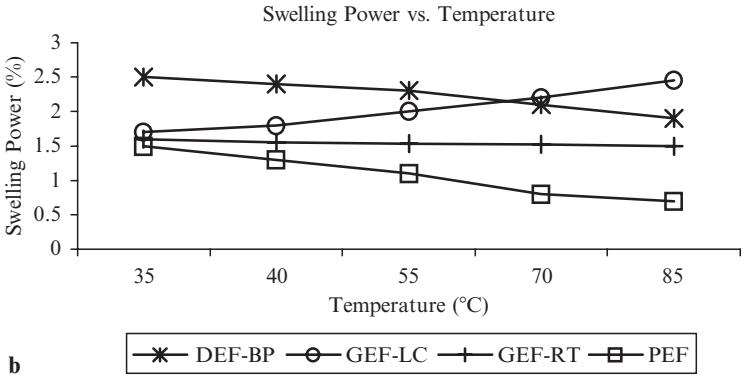


Fig. 4.2 (continued)

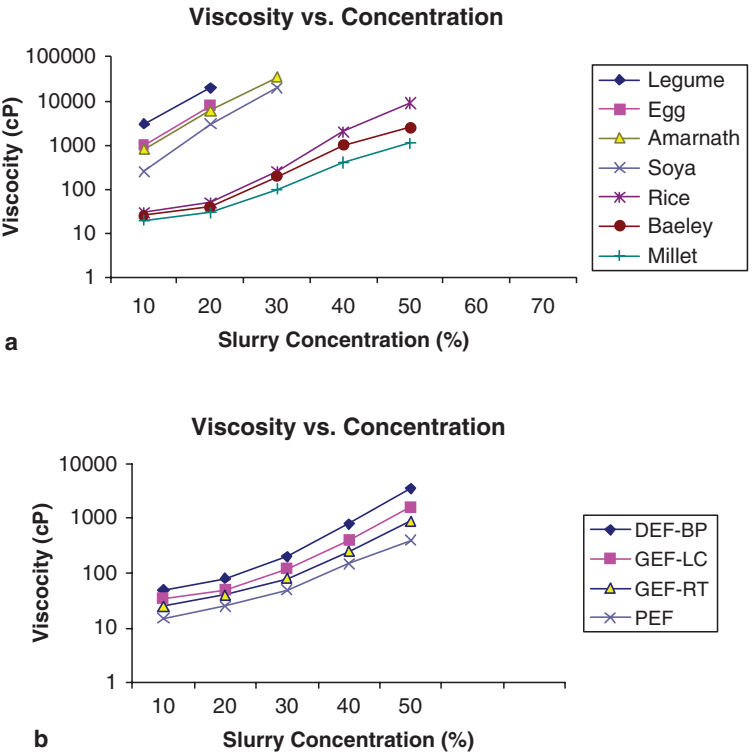


Fig. 4.3 **(a)** Viscosity of ingredients, (**b**) Viscosity of enteral foods

food formulations were considerably lower than the proprietary enteral foods based on chemically defined ingredients at identical solid concentrations. The osmolalities and RSLs of a few enteral foods are shown in Table 4.8 for reference.

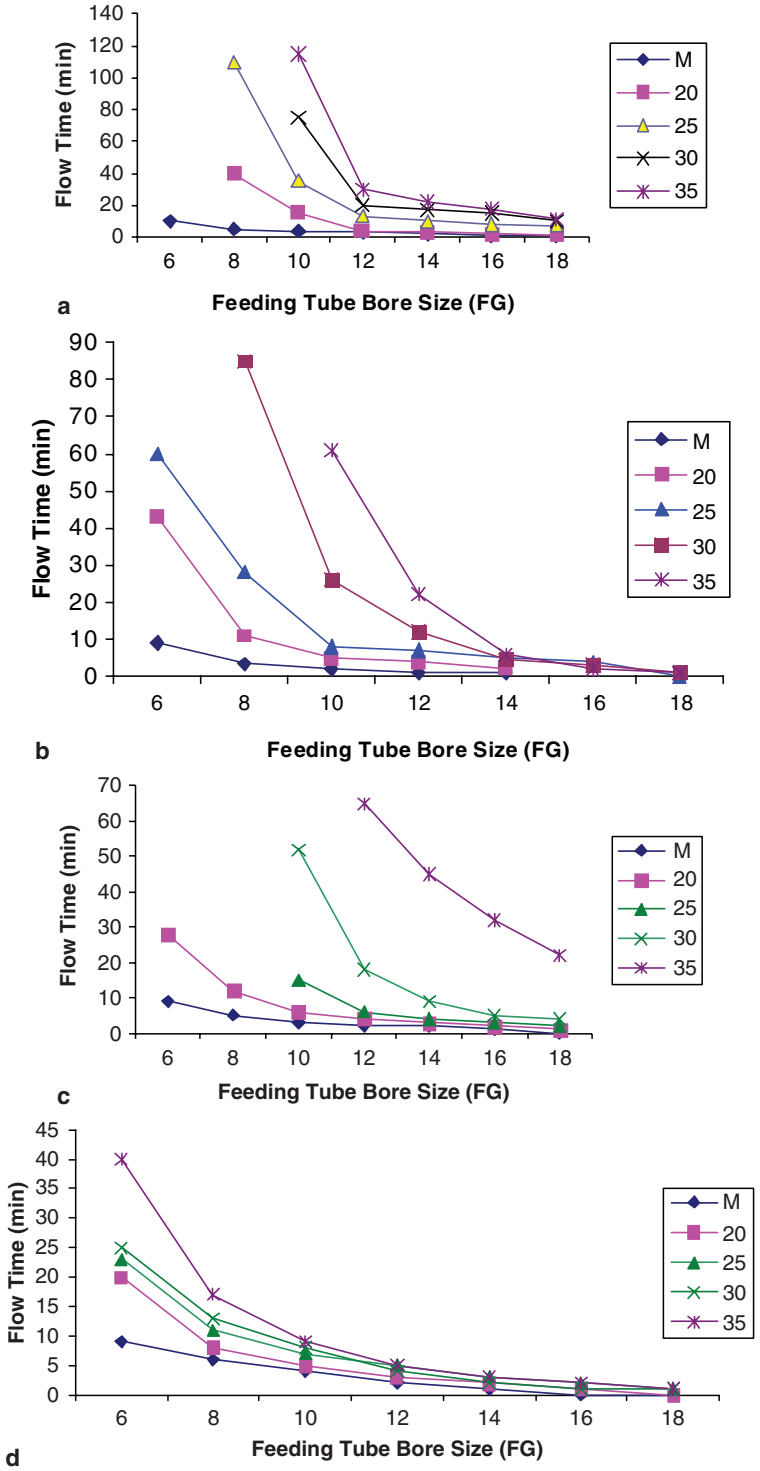


Fig. 4.4 Flow Characteristics of Enteral Foods, (**a**) GEF-LC, (**b**) GEF-RT, (**c**) DEF-BP, (**d**) PEF

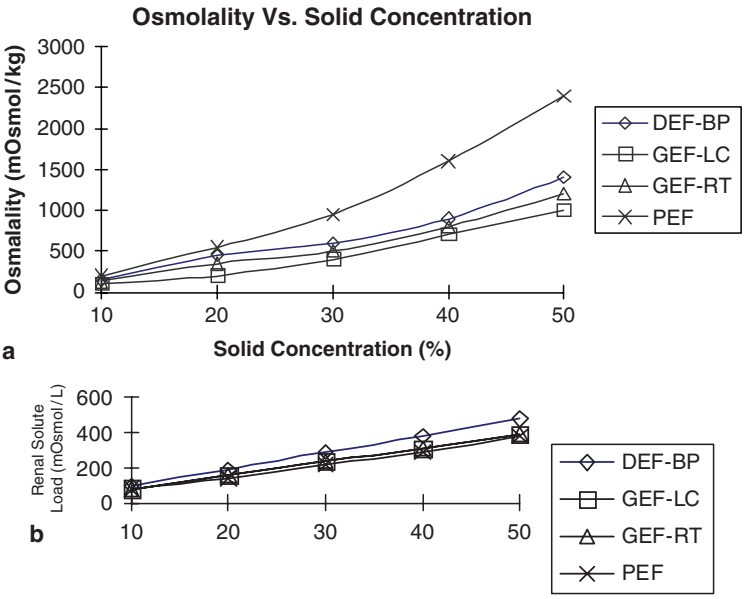


Fig. 4.5 **(a)** Osmolality of enteral foods, (**b**) Renal solute load (RSL) of enteral foods

Table 4.8 Osmolality and renal solute load (RSL) of some proprietary enteral foods

Enteral foods	Intended use	Osmolality (mOsmol/kg water)	RSL (mOsmol L^{-1})
Jevity 1.0[a]	Long-term tube feeding	300	370
Pulmocare[b]	COPD, cystic fibrosis	475	512
Glucerna[a]	Type I and II diabetes	355	360
Promote[a]	Wound healing pressure ulcer	380	499
Peptamen[c] (vanilla)	Pancreatitis transition from TPN	380	319
Nutrihep[c]	Hepatic disease	790	301
Similac[a]	Infant with colitis	370	171
Pediasure[a]	1–13-years old	355	271

[a]www.ross.com (product hand book)
[b]Novartis Medical Nutrition, Pocket Guide, 2005
[c]Nestle Nutrition, Pocket Guide, 2007

The ethanol extracTable sugar contents in the raw blend of low-cost enteral food were 6.2%, whereas the same food when cooked contained almost four times (23% dwb) higher levels of free sugars than the raw blands. This could have happened due to the hydrolysis of starch by the malt enzymes during cooking (Malleshi et al., 1986). On the other hand, the free sugar contents of spray-dried foods, namely GEF-RT and DEF-BP, were 21.2 and 20.6%, respectively. Glucose, fructose, and maltose were the main constituent sugars of the ethanol extracts of the foods.

The gel permeation chromatograms of the carbohydrates of GEF-LC, GEF-RT, DEF-BP, pure maltodextrin, a maltodextrin-based proprietary food (PEF), and the unmalted blend of GEF-RT and DEF-BP are presented in Fig. 4.6a–c. It can be observed that the major amounts of carbohydrates of the enteral foods are comparable with maltodextrin of 12–15 dextrose equivalents. The molecular weights of the major fractions of carbohydrates of GEF-LC, GEF-RT, and DEF-BP were 21,000, 14,000, and 15,000, respectively, which was higher than that of PEF (12,000).

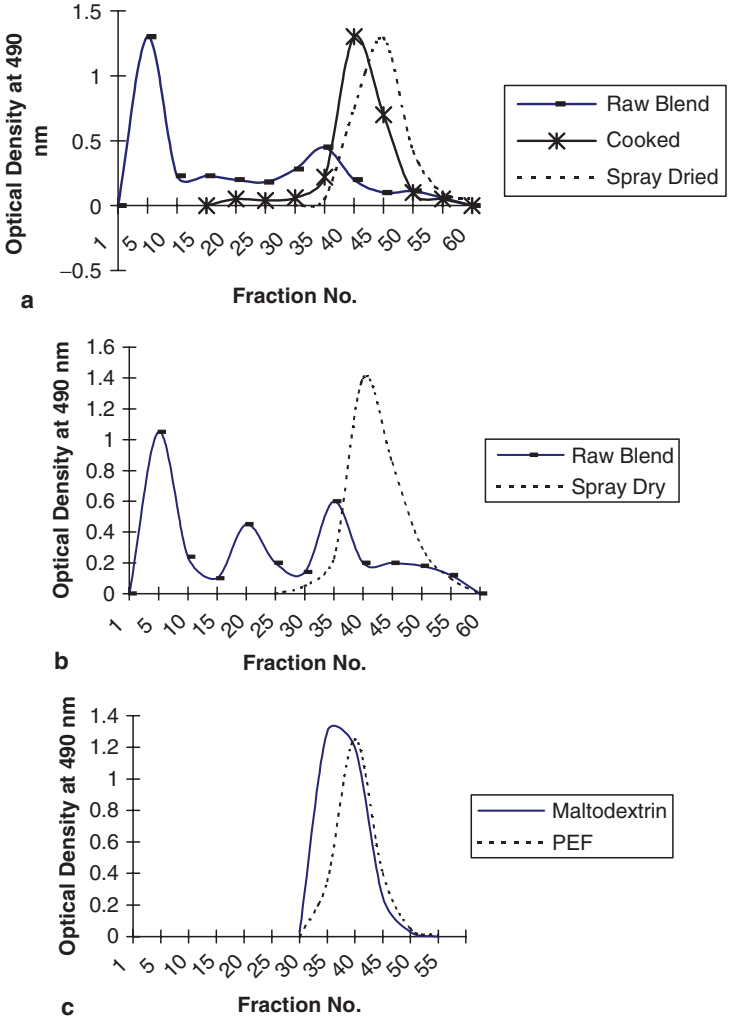


Fig. 4.6 (**a**) Gel permeation chromatograms of raw blend, cooked blend (LCEF), and Spray Dried enteral foods (GEF-RT), (**b**) Gel permeation chromatograms of DEF-BP, (**c**) Gel permeation chromatograms of maltodextrin and PEF

On the other hand, the food prepared by blending unmalted ingredients of GEF-RT and DEF-BP exhibited the chromatogram with a prominent void volume peak and much smaller second peak. This clearly shows that the carbohydrates of the unmalted blends were not predigested whereas that of its malted counterparts (formulated enteral foods) were fully predigested.

The amino acid contents of the enteral foods, egg, and milk, specifically arranged as other amino acids, aromatic amino acids, branched chain amino acids, and sulfur amino acids are presented in Tables 4.9 and Table 4.10 and in Fig. 4.7. The lysine content of GEF-LC (6.10%) was higher than in DEF-BP (5.04%) and GEF-RT (4.73%). Probably spray drying caused slight reduction in the lysine content of GEF-RT and DEF-BP. Likewise, a slight difference with respect to glutamic acid, aromatic amino acids, branched chain amino acids as well as sulfur amino acid contents also existed among the foods.

The protein profile as determined by SDS-PAG electrophoresis (Fig. 4.8) reveals that the bands corresponding to the proteins of the individual ingredients corresponded with that of enteral food proteins but with slightly reduced intensity. This

Table 4.9 Amino acid content of the enteral foods (gram per 100 g of protein)

Amino acids	GEF-LC	GEF-RT	DEF-BP
Other amino acids			
Aspartic acid	10.60	10.39	9.79
Serine	5.85	5.32	6.08
Glycine	4.77	5.27	5.07
Histidine	3.07	2.85	2.52
Arginine	2.70	3.19	3.22
Threonine	4.23	3.96	4.20
Alanine	4.64	4.50	4.93
Proline	8.01	7.22	8.15
Lysine	6.10	4.73	5.04
Tryptophan	1.24	1.18	1.27
Glutamic acid	25.35	23.27	20.74
Aromatic amino acids (AAA)			
Tyrosine	0.53	2.23	2.05
Phenylalanine	6.38	7.16	6.98
Total AAA	6.91	9.39	9.03
Branched chain amino acids (BAAA)			
Leucine	6.22	6.08	5.92
Isolucine	2.88	2.43	2.73
Valine	5.98	3.37	4.68
Total BCAA	15.08	11.88	13.31
Sulfur amino acids (SAA)			
Cystine	0.53	0.69	0.46
Methionine	2.73	2.48	2.65
Total SAA	3.23	3.15	3.40

Table 4.10 Amino acid content of egg and milk

Amino acids	Egg	Milk
Other amino acids		
Aspartic acid	7.31	7.67
Serine	7.02	5.50
Glycine	3.46	2.17
Histidine	2.40	2.28
Arginine	6.69	2.86
Threonine	5.19	4.31
Alanine	5.91	3.54
Proline	4.15	9.42
Lysine	7.15	7.09
Tryptophan	1.48	1.42
Glutamic acid	12.34	20.71
Aromatic amino acids (AAA)		
Tyrosine	3.95	4.62
Phenylalanine	5.78	4.30
Total AAA	9.72	8.92
Branched chain amino acids (BAAA)		
Leucine	7.80	9.71
Isolucine	6.75	6.24
Valine	7.39	5.50
Total BCAA	21.94	21.45
Sulfur amino acids (SAA)		
Cystine	2.11	0.70
Methionine	3.42	2.56
Total SAA	5.53	3.26

From FAO (1968)

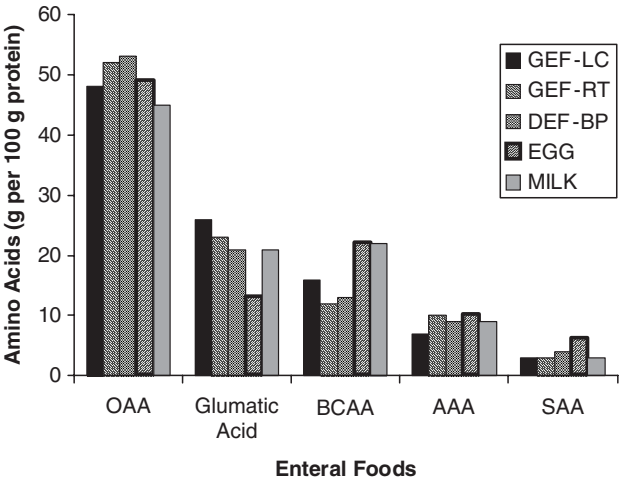


Fig. 4.7 Amino acid content of enteral foods, egg, and milk

indicates that processing caused a little change in the molecular pattern of the proteins of the ingredients. The molecular weights of the proteins of ingredients were of 15–45 kD.

The protein patterns of the native rice, barley, finger millet, mung bean, and their malts, amaranth and popped amaranth presented in Fig. 4.8 show that the major qualitative changes during malting occurred in mung bean proteins. The protein fraction, namely 45–55 kD of the malted mung bean, was hydrolyzed to a larger extent by the malt proteases into lower molecular weight proteins, and out of those, the bands of 40 and 29 kD were prominent. The intensities of the bands of 45–55 kD in malted mung bean are also very feeble. On the other hand, the changes in the protein fractions of rice, barley, and finger millet on malting and also of amaranth on popping were very marginal. This was expected, because the proteases in sprouted cereals are less active as compared with that of legume sprouts.

The fatty acid profiles of some fats and oils and that of the enteral foods are presented in Table 4.11 and Table 4.12, respectively. Nearly, 65% of the lipids of enteral foods were made up of unsaturated fatty acids. Linoleic acid was the major fatty acid in the lipids of the GEF-LC and GEF-RT (34%), whereas oleic acid was the major fatty acid present in DEF-BP (30%), which are shown in Fig. 4.9a, b. A total of 5.9% n-3 PUFA (2.7% eicosapentaenoic acid–EPA and 3.2% docosahexaenoic acid-DHA) was present in the lipid of DEF-BP (Fig. 4.9b). The cholesterol and phospholipids contents of the GEF-LC and GEF-RT were 400, 970 and 340, 970 mg per 100 g of lipids, respectively, whereas that of DEF-BP was 1.4 and 6.9 g per 100 g of lipids. The higher level of cholesterol as well as phospholipids in DEF-BP could be contributed by egg content of the food. The major source of cholesterol in

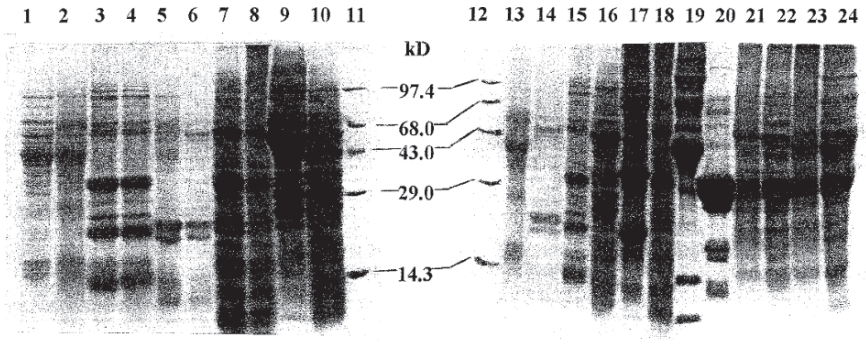


Fig. 4.8 SDS-PAGE pattern of native and processed ingredients and enteral foods

GEF-LC and GEF-RT is from milk. Mashing and spray drying did not affect the cholesterol content of the foods.

The microbial profile of the fresh enteral foods is presented in Table 4.13. The total plate count of GEF-LC was 7.1×10^6 cfu g^{-1}, and about 72% of the mesophilic aerobes were LAB. On the other hand, nearly 90% of the mesophilic aerobes of GEF-RT and DEF-BP were LAB. The counts for yeast and mold and the mesophilic spore formers were very marginal. The coliform MPN index was negligible. The *E. coli* count was just 3.3×10^3 cfu g^{-1}. *Streptococcus aureus*, *salmonella, B. cereus*, and *fecal streptococci* were not detected in all the foods. Although the total plate count (TPC) was much higher than the limits prescribed for the nutritious foods (ISI, 1985), there was no reason for concern as the major counts were LAB obtained from the pure culture of yogurt. The small levels of coliform and spore-forming bacteria in the foods may be from the water used for the preparation of the foods.

The microbial profile of the foods at various stages of their preparation was also evaluated. It was observed that the cold water slurry of the ingredients exhibited very

Table 4.11 Fatty acid profile of some fats and oils

Fatty acid	Cow's milk fat[a]	Cod liver oil[a]	Egg Yolk lipid[a]		Soy oil[a]
	4:0	3	–	–	–
	6:0	2	–	–	–
	8:0	1	–	–	–
	10:0	3	–	–	–
	12:0	4	–	–	Tr
Myristic	14:0	12	1	–	Tr
	14:1	1	–	–	–
Palmitic	16:0	26	19	29	10
Palmitoleic	16:1	3	4	4	Tr
Stearic	18:0	11	5	9	4
Oleic	18:1	28	15	43	25
Linoleic	18:2	2	2	11	52
Alpha-linolenic	18:3	1	–	–	7
	20:0	–	–	–	Tr
	20:1	–	10	–	–
Arachidonic	20:4	–	1	–	–
EPA	20:5	–	6	–	–
	22:0	–	4	–	Tr
	22:1	–	2	–	–
DHA	22:6	–	27	–	–
Others	3	4	4	2	
Cholestrol	0.2–.04[b]	0.57[b]	1.6[b]	–	
Phospholipids	0.2–1.0[b]	–	14[b]	–	
Unsaturation index Ψ	39	23	69	150	96.6
P/S	0.05	1.24	0.81	4.21	

Unsaturation index Ψ was calculated by summing up of percent of individual unsaturated fatty acid multiplied by corresponding number of double bonds

P/S. Polyunsaturated fatty acids/saturated fatty acids

[a]Garton (1993)

[b]Gunstone and Padley (1997)

Table 4.12 Fatty acid profile of enteral foods

Fatty acid		GEF-LC	GEF-RT	DEF-BP	
	4:0	–	–	–	
	6:0	–	–	–	
	8:0	–	–	–	
	10:0	–	–	–	
	12:0	–	–	–	
Myristic	14:0	7.41	6.96	6.0	
	14:1	–	–	–	
Palmitic	16:0	20.85	20.99	21.25	
Palmitoleic	16:1	3.25	2.76	4.52	
Stearic	18:0	8.39	8.41	8.12	
Oleic	18:1	26.81	27.81	30.92	
Linoleic	18:2	33.29	33.07	17.43	
Alpha-linolenic	18:3	–	–	1.54	
	20:0	–	–	–	
	20:1	–	–	–	
Arachidonic	20:4	–	–	4.36	
EPA	20:5	–	–	2.65	
	22:0	–	–	–	
	22:1	–	–	–	
DHA	22:6	–	–	3.20	
Others		–	–	–	
Cholestrol		0.40	0.34	1.39	
Phospholipids		0.97	0.97	6.91	
Unsaturation index Ψ		96.6	97.70	124.8	
P/S			0.91	0.91	0.82

Unsaturation index Ψ was calculated by summing up of percent of individual unsaturated fatty acid multiplied by corresponding number of double bonds

P/S. Polyunsaturated fatty acids/saturated fatty acids

[a]Garton (1993)

[b]Gunstone and Padley (1997)

high count, not only for the mesophilic spores (11×10^5 cfu g^{-1}) but also for yeast and mold (7×10^1 cfu g^{-1}), coliforms (17×10^2 cfu g^{-1}), mesophytic spore formers (7×10^3 cfu g^{-1}), and staphylococci (17×10^2 cfu g^{-1}). However, cooking the slurry destroyed completely the yeast, mold, and most of the mesophilic spore formers, which brought down the total plate count (TPC) considerably. When the pure culture of LAB was added to the slurry, homogenized, and spray dried, nearly 60–70% of the LAB survived the spray drying process. The low-cost food was also microbiologically safe, after cooking and cooling the food. Nearly 50% of the added LAB of the GEF-LC survived the cooking process. Hence, the food was still enriched with beneficial microflora even on cooking. In the absence of specialized standards for microbial load of enteral foods, the microbial load recorded in the present study may be considered as safe and permissible. Generally, it is suggested that the maximum TPC for most of the nutritious food products should be 50,000 g^{-1} of food, and the maximum permissible coliform count should be 10 g^{-1}. With regard to coliform count, the enteral foods were also well within the permissible level as their coliform was only 2 g^{-1}.

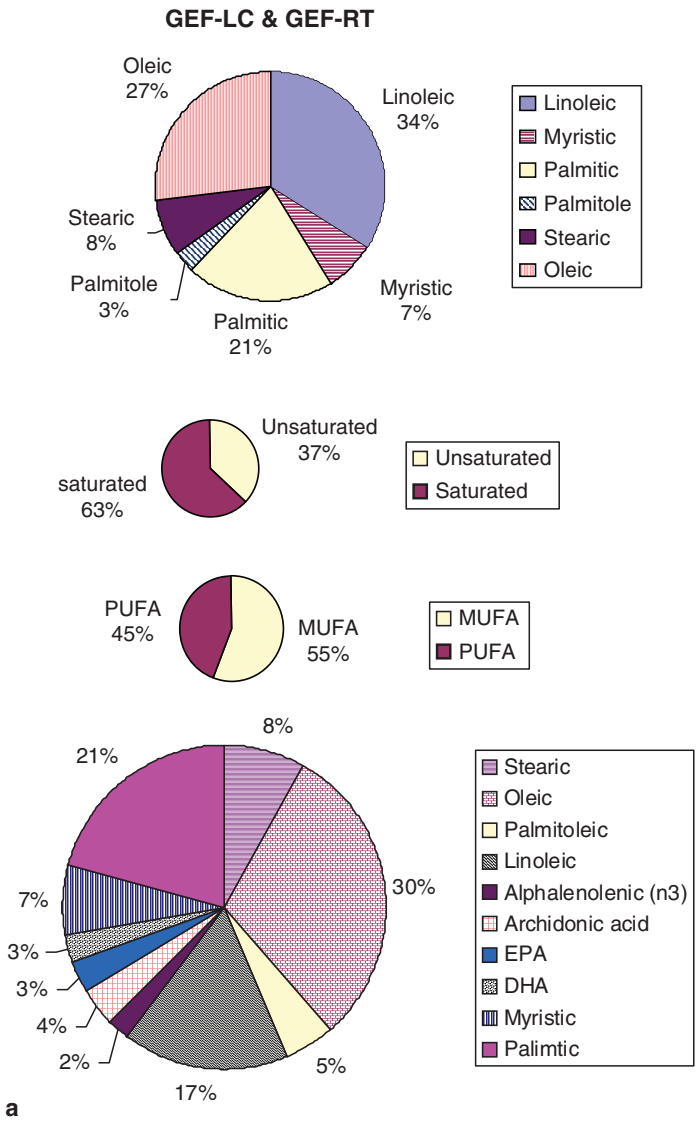


Fig. 4.9 Fatty acid profile of enteral food lipids, (a) GEF-LC and GEF-RT, (**b**) DEF-BP

The microbial load of the reconstituted foods in water when fresh and after keeping at room temperature (25 C) up to 12 h was monitored and recorded on hourly basis. The results indicated that the foods remained microbiologically safe up to about 6 h of preparation when left at room temperature (Tables 4.14 and Table 4.15). Therefore, the foods can be prepared at hospitals twice a day

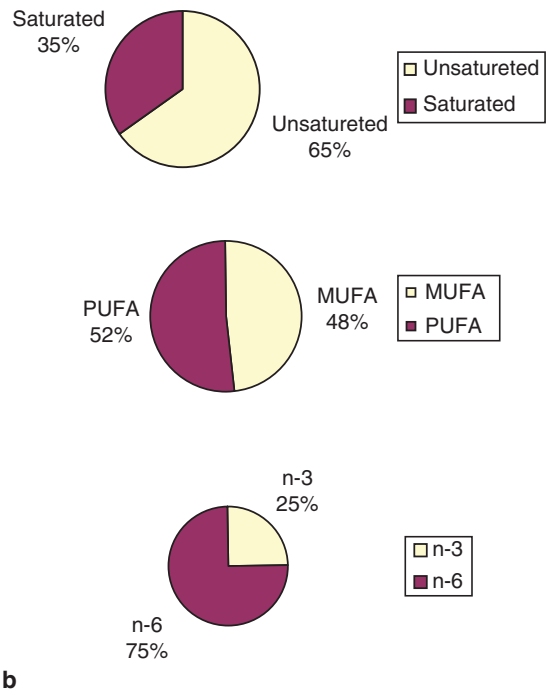


Fig. 4.9 (continued)

Table 4.13 Microbial Profile of Fresh Enteral Foods

	Microflora (log_{10}cfu g^{-1})			
Enteral foods	Mesophilic aerobes	LAB	Yeast and mold	Mesophilic spore form
GEF-LC	7.1	5.2	3.0	3.9
GEF-RT	5.8	5.5	0.8	4.6
DEF-BP	6.0	5.4	2.0	4.3

and the patients can be fed within 6 h of preparation of the food. This minimizes the frequency of preparation of the food for feeding in the hospitals. The food can be prepared even less frequently in those hospitals having the facilities to pack and store the prepared food in cold temperature (0 –4°C). One interesting observation was that GEF-LC remained safe at room temperature up to12 h of preparation. Direct cooking of GEF-LC might have killed the harmful microorganisms contributing to the extended periods of safety. The foods were free from most of the pesticide residues. However, the very small levels of g-BHC detected (0.005 ppm) were also within the permissible level (0.05 ppm) for its contents in such foods (PFA, 1996).

Table 4.14 Time course study on microbial quality of ready-to-serve enteral foods at room temperature of 25°C

Sample	Mesophilic aerobes (cfu g^{-1})	LAB (cfu g^{-1})	Yeast and Mold count (cfu g^{-1})	Coliforms (Mc Conkey agar)
GEF-LC				
h_0	2×10^3	3×103	Nil	−ve
h_2	2×10^3	3×103	Nil	−ve
h_4	2×10^3	2×103	Nil	−ve
h_8	4×10^3	1×103	Nil	−ve
h_{12}	10×10^3	4×103	Nil	−ve
GEF-RT				
h_0	9×10^3	7×103	Nil	−ve
h_2	16×10^3	8×10^3	Nil	−ve
h_4	15×10^3	13×10^3	Nil	−ve
h_8	716×10^3	346×10^3	Nil	+ ve
h_{12}	200×10^3	203×10^3	Nil	Heavy
DEF-BP				
h_0	73×10^3	2×10^3	Nil	−ve
h_2	88×10^3	13×10^3	Nil	−ve
h_4	98×10^3	14×10^3	Nil	−ve
h_8	200×10^3	225×10^3	Nil	+ ve
h_{12}	676×10^3	Heavy	Nil	Heavy

h_0, h_2, h_4, h_8, and h_{12} represent hours after reconstitution of foods in water

Table 4.15 Time course study on microbial quality of ready-to-serve enteral foods at room temperature of 25°C

Sample	Coliforms (EMB agar)	*B. cereus*	Staphylococcus
GEF-LC			
h_0	−ve	−ve	−ve
h_2	−ve	−ve	−ve
h_4	−ve	−ve	−ve
h_8	−ve	–	−ve
h_{12}	−ve	−ve	−ve
GEF-RT			
h_0	−ve	−ve	−ve
h_2	−ve	−ve	−ve
h_4	−ve	−ve	−ve
h_8	−ve	−ve	−ve
h_{12}	Heavy	+ve	15×10^1
DEF-BP			
h_0	−ve	−ve	−ve
h_2	−ve	−ve	−ve
h_4	−ve	−ve	−ve
h_8	+ ve	+ ve	+ ve
h_{12}	Heavy	Heavy	9×10^1

h_0, h_2, h_4, h_8, and h_{12} represent hours after reconstitution of foods in water

Discussion

The soluble fiber of the enteral foods is mainly extracted from b-glucans of barley, whereas the insoluble fiber is derived from mung bean, soy polysaccharides, and finger millet malts (Malleshi, 1992). The role of dietary fiber in human colon has been discussed (Cuminngs et al., 1980; Rays et al., 2005), wherein they indicated that the water holding capacity of fiber, production of short-chain fatty acids in colon, and fiber-induced alterations in colonic microflora all have beneficial effects on gastrointestinal tract and colonic functions. The beneficial role of dietary fiber in enteral foods has been well recognized. Nowadays, many of the defined formulations also contain quite good amount of dietary fiber. Soy polysaccharides are the primary source of dietary fiber in most of the enteral foods (Mark and Stalker, 1993). The dietary fiber component of enteral foods undergoes microbial fermentation to produce short-chain fatty acids (SCFA), which happens to be the sole fuel for intestinal villi, and thus play a very important role in maintaining villus architecture (Silk, 1993; Roy et al., 2004; Topping, 2007). The role of dietary fiber in managing hyperglycemia, hypercholesterelemia, and preventing colon cancer and constipation among others is well discussed (Kahlon and Chow, 1997; Thebaudin et al., 1997; Slavin, 2005; Harshman and Aldoori, 2006).

The protein and energy contents of disease-specific enteral food were much higher than that of the general-cagory enteral foods. It may be termed as high-protein food. One-thousand milliliters of the food at tube feeding consistency provide around 60–70 g protein and 1,200–1,800 kcal. Besides adequate quantity of dietary fiber (15 g L^{-1}), the DEF-BP provides enhanced levels of vitamin A, C, D, E, calcium, magnesium, and zinc. The burn patients are at hypercatabolic state and need high protein and high-energy diet. Therefore, often the enteral foods for burn patients are prepared as modular diet by adding extra quantity of amino acids and lipids to increase the protein and energy content (Glottschlich et al., 1990). The DEF-BP derives high protein as well as high energy from the natural foods, mainly from eggs, milk, cereals, grain legumes, and soy oil. Because both milk and eggs provide intact proteins, their physiological acceptance and sensory acceptance will be higher than the formulae containing free amino acids or hydrolyzed protein (Cope et al., 1995; Bhutta et al., 2004).

The role of glutamine and arginine has been well established as conditionally EAA for patients of special clinical conditions (Gennari et al., 1995). They are known to enhance the immune functions (Panigrahi et al., 1997; Peng et al., 2006), prevent bacterial translocations (Gianotti et al., 1995), and serve as specific fuel also. Therefore, the glutamine content of the enteral foods was increased by about 3.5% of the total amino acids. Branched chain amino acids (BCAA) are the major skeletal muscle proteins, which help localized metabolism besides improving the liver function (Ferrando et al., 1995; Ferenci, 1996). In addition, recently, Kakazu et al. (2007) have reported the immune-enhancing effect of BCAA in cirrhotic patients. The enteral foods formulated in the present study contained an appreciable amount of BCAA (13–15 g/100 g of protein) from natural sources such as mung bean, egg, and milk. The role of arginine in wound healing and improving the

immune status has been reported (Barbul, 1986; Patel, 2005). The arginine content of DEF-BP was 3.2% of total amino acids. Fish oil is a good source of n-3 fatty acids and is known to be an immune-enhancing component. It has been reported that fish oil helps maintain the intestinal permeability (Turley and Strain, 1993; Wild et al., 1997; Bancroft et al., 2003; Kreymann et al., 2006), which normally undergoes disruption following burn injury. Hence, the lipids of DEF-BP were enriched with fish oil.

The presence of lactics especially the LAB in the food has several advantages such as suppression of pathogenic microflora in the intestine and development of beneficial microflora (Gilliland and Speck, 1977; FAO/WHO, 2001; Bengmark, 2005). Besides, the LAB are known to help overcome lactose intolerance due to the breakdown of lactose in the alimentary tract to its structural components, namely glucose and galactose (Alm, 1991; FAO/WHO, 2001). In recent years, acidophilus milk has gained importance. Several reports indicate that the presence of LAB in foods helps prevent bacterial translocation, enhances the immune status, maintains mucosal barrier, and improves the palatability of the foods (Berg, 1992; Patidar and Prajapati, 1997; Bengmark, 2005). More recently, many investigators have reported the beneficial effects of probiotics such as LAB and dietary fiber (together termed as synbiotics) in maintaining good health besides preventing and/or managing many diseases (FAO/WHO, 2001; Chakravarty et al., 2002; Bengmark, 2005)

The simple way of blending different ingredients to prepare low-cost enteral foods was effective, as the food had natural taste, aroma and was of uniform texture. Because the ingredients were in dry state, the interaction amongst the nutrients was also minimal. The slurry thinned down when cooked, indicating that the hydrolysis of starch by the malt enzymes occurred effectively, and that the enzyme activity of the malt was not affected by the presence of other ingredients of the foods. This concept of developing low-cost enteral foods based on indigenous ingredients is expected to be useful to provide cost-effective nutritional support to the patients in developing countries.

The technology adopted for the preparation of precooked, ready-to-eat enteral foods involves slow cooking of the malt that facilitates gelatinization of starch and subsequently hydrolysis of the starch to simple sugars and dextrins (Berfort and Heriksnas, 1983; Chakravarty et al., 2002). Cooking the malt in water containing milk or eggs did not affect the enzyme activity of malt, as the starch hydrolysis was completed on cooking. However, while cooking, the egg proteins coagulated and the milk curdled. This necessitated homogenization of the cooked food before spray drying. The cooked food of about 30% solid concentration and 140-cP viscosity was spray dried without any kind of obstruction. The spray-dried product was of creamy white color, smooth texture, and of free-flowing nature. Cooking the ingredients together probably had some technological advantages with regard to the textural modification of the egg protein. The presence of milk fat, egg fat, and other externally added vegetable oils and hydrolyzed carbohydrates might have interfaced with the egg protein moieties, preventing irreversible coagulation of egg protein upon heating to a larger extent. It is believed that the gentle spray drying of egg yolk prevents heat abuse and contributes to the emulsion stability by steric and

particle mechanism. Further, mechanical force applied during homogenization of the cooked mass containing hydrolyzed starch, partially hydrolyzed protein, fiber, and partially coagulated egg protein in a colloidal mill would have ruptured the protein structure and denatured its agglomeration characteristics. Because of this reason, the egg protein in the DEF-BP may remain in a free-flowing state without coagulation even on heating. It has been reported that small starch granules like that of rice (Champagne, 1996) and popped amaranth (Walkowski et al., 1997) aggregate into the porous sphere when spray dried with proteins or water-soluble polysaccharides and act as a fat mimetic. This may also play an important role in maintaining smooth texture of the foods in dry form and stable emulsion of the reconstituted slurry without phase separation or coagulation even after leaving up to 6 h. The gum acacia, which formed a small constituent of the foods, might have also helped in maintaining stability of the emulsified foods on reconstitution (Chanamai and McClements, 2002). It has also been reported that gum Arabic products protect spray-dried flavor against oxidation (PCR, 1993).

Most of the literature regarding the use of egg proteins in food formulations concentrates on its coagulation characteristics upon heating. Literature relating to the prevention of coagulation characteristics of egg protein following heat treatment is scanty. The observations made in the present study wherein the food containing the egg continues to be in the free-flowing state even on heating could be the first of its kind. Indeed, it deserves detailed investigation on the nature of the changes the egg protein has undergone during processing. The innovative process of preparing a spray-dried product based on cereals, milk, and egg has its potential for application to prepare high valued egg-based products, such as health foods, sport foods, and food for special dietary uses.

When the enteral feeding is delivered orally, the consistency of the food may not be very critical as it can be a semisolid paste that can be spoon-fed. But when the food is to be fed through a tube, there is a threshold limit for the thickness or the viscosity of the food. The enteral food having consistency similar to milk (about 60 cP) may pass through a feeding tube of as small as 6-FG bore size easily. But when the total fluid intake becomes a limiting factor, as in the case of renal failure (Beto, 1995), liver failure, the food should be enriched with calorie so that it provides 1.5–2 kcal mL^{-1} food, and still be suitable for tube feeding. In such a situation enzyme-rich, fiber-regulated malt flour from cereals could be gainfully utilized to develop a range of enteral foods akin to normal wholesome diet.

Continuous drip feeding has certain advantages over selected bolus feeding in terms of tolerance and acceptability, especially with the patients in ICU, and patients with short bowel rejections. The flow behavior of the food formulations developed in the present study indicates that reconstituted foods with a minimum of 0.7 kcal mL^{-1} to about 2 kcal mL^{-1} are suitable for delivering as continuous drip feeding, bolus, or oral feeding as the situation demands.

As discussed earlier, the osmolalities of the enteral foods have significant effects on the tolerance and overall acceptability as well as the effectiveness of the enteral nutrition support. The acceptable level of osmolality for such type of foods ranges from 300 to 650 mOsmol kg^{-1} that can be delivered in full strength to the patients.

However beyond this osmostic load, an adaptation period of 24–72 h may be required (Mahan and Arlin, 1992). The favorable osmolality of the enteral foods developed in the present study may be attributed to the low degree of starch hydrolysis and partial hydrolysis of intact protein as compared with the chemically defined enteral foods where high levels of osmotically active particles (sugars, amino acids, salts, etc.) are present (Smith and Heymsfield, 1983; Nalin et al., 2004). The RSL calculated was also considerably lower for the enteral foods formulated compared with several proprietary foods. However, unlike osmolality, RSL is contributed by mineral and protein contents only.

Although RSL has to be suitably restricted in case of patients with renal failure and for pediatric patients, care has to be taken to control RSL for general-cagory patients also, so that it does not cause excessive load on the kidney. Most of the general-cagory enteral foods prepared by blending hydrolyzed protein, maltodextrin, and other ingredients exert RSL higher than the desirable level. The lower osmolality as well as the RSL of the malt-based enteral foods permit to provide high concentration of the nutrients (up to 30–35% solid contents) without causing any adverse consequences.

From these observations, it is evident that malted cereals and grain legume could be gainfully utilized to develop nutritionally balanced, energy-dense, and cost-effective enteral foods.

Summary and Conclusion

Malt flour from rice, barley, finger millet, mung bean, defatted and toasted soy, whole milk powder, and soy oil were blended in suitable proportion, and the blend was fortified with macro and micronutrients, taurine, carnitine, conditionally essential nutrients, and LAB. The food thus prepared was termed as GEF-LC. This food requires cooking prior to feeding. The blend, consisting of cereal malts, legume malt, soy flour, and soy oil was mixed with fresh pasteurized milk and potable water. The slurry was cooked and cooled to room temperature and to that, yogurt containing live LAB was added. The contents were homogenized and spray dried to prepare GRF-RT. The cereal and legume malts, pasteurized milk, fresh whole egg, popped amaranth, fish oil, and soy oil were processed similar to GEF-RT to prepare DEF-BP. Enteral foods thus prepared were fortified with vitamins, minerals, and conditionally essential nutrients.

The physicochemical characteristics of the enteral foods were in line with the internationally followed guidelines for such foods. The general-cagory food contained 16% protein and 12% fat, whereas the disease-specific food contained 21% protein and 19% fat. One-thousand milliliters of the enteral foods at tube feeding consistency provide around 40–65 g protein, 1,000–1,600 kcal, and 10–15 g dietary fiber. The foods reconstituted well in water, milk, or any other potable liquids, and retained uniform consistency up to 6 h after preparation. The foods at 20–30% solid contents had smooth flow behavior through 6–18-FG bore feeding tubes.

The osmolalities of the foods at 25–30% solid contents ranged form 246 to 500 mOsmol kg^{-1} and their RSLs varied from 195 to 219 mOsmol L^{-1}. The pesticide residue content of the foods was well within the permissible limits for such specialty foods. All the three foods were microbiologically safe and contained active probiotics namely LAB.

Processing the ingredients for preparation of the enteral foods caused drastic changes in the carbohydrate profile, but marginal changes in the protein profile. The molecular weights of the carbohydrates were in the range of 15,000–21,000, whereas the proteins were of 15–45 kD. The food proteins contained highly desirable proportion of BCAA, SAA, and AAA for enteral foods. Nearly 65% of the food lipids were made up of PUFA, and major portion of them were oleic and linoleic acids. The ratio of n-6 to n-3 PUFA in the disease-specific enteral food was 3:1. Sixty to 70% of the LAB survived spray drying.

In conclusion, malted cereals and grain legumes could be gainfully exploited to prepare nutritionally balanced and cost-effective enteral foods.

Chapter 5
Protein Quality Evaluation of Enteral Foods

Introduction

This chapter describes the protein quality evaluation of the enteral foods developed in this project. The protein quality of a food product not only depends on the nature of ingredients used, but also on the processing method followed. Although nutrient losses during processing can be minimized by optimizing processing methods, some amount of nutrient losses specially that of heat-sensitive nutrients are inevitable. Studies have shown the losses of certain amino acids such as lysine and other amino acids during heat treatment, thereby lowering the protein quality (Ilo and Berghofer, 2003; Yeung et al., 2006). Studies have also suggested supplementation of amino acids in infant food and enteral foods to preserve the protein quality (Sarwar et al., 1993; Sarwar and Peace, 1994). The food formulations based on cereals, legumes, and proteins of animal origin that are blended in suitable proportion and fortified with vitamins and minerals may generally be termed as nutritionally balanced foods. However, considering the processing loss, it is suggested that the nutritional quality of a food protein should be evaluated by in vitro test as well as be confirmed by an appropriate in vivo test (Vanderveen, 1981). Hence, it is customary that whenever a new food is formulated, its nutrient analysis, growth-promoting quality, and related aspects of protein quality are determined by animal feeding trials (Pellet and Young, 1980; Sarwar and Peace, 1994). Usually, protein efficiency ratio (PER) of the food is determined in weanling rats, and metabolic studies are carried out on growing rats. The data generated from the PER and metabolic studies are usually utilized to evaluate the nutritional compatibility of the food protein for humans (FAO, 1991).

Generally spray-dried and roller-dried malt foods have slightly lower PER than the malted cereals and legume-based foods (Rao et al., 1976; Wondimu and Malleshi, 1996) that need cooking prior to feeding. This might be due to the Maillard Reaction – the reaction between malt sugar and amino acids – that occurs during spray drying, and thus resulting in decreased bioavailability of lysine (Dworschank, 1980; Lowry et al., 1990, Yeung et al., 2006). Any damage to the essential amino acids such as lysine, methionine among others is reflected in low biological value of the proteins. It is desirable that the enteral food proteins should

M. Kaur, *Medical Foods from Natural Sources,*
DOI: 10.1007/978-0-387-79378-8_5, © Springer Science+Business Media, LLC 2009

be of high biological value. Although the main ingredients of enteral foods in the present study are malted cereals, legumes, milk, and egg, it was interesting to know the nutritional quality of the food protein, because these ingredients had further undergone processing. Hence, the protein quality of the enteral foods was evaluated by chemical analyses (in vitro) of its amino acid profiles as well as by conducting animal feeding (in vivo) experiments. The details of the chemical analyses of amino acid profiles of the enteral food proteins are discussed in Chap. 4. The protein quality as assessed by protein efficiency ratio (PER), net protein ratio (NPR), biological value (BV), true digestibility (TD), net protein utilization (NPU), and protein digestibility corrected amino acids score (PDCAAS) conducted in animal models is presented in this chapter.

Materials and Methods

Protein Quality Evaluation by Animal Experimentation

The protein quality of the enteral food protein was evaluated by following the standard procedure as discussed later. For the purpose of comparison, the protein quality evaluation of a proprietary enteral food (PEF) was also carried out simultaneously.

Composition of the Diets

The composition of the diets of enteral foods and the reference protein to contain 10% protein and the nonprotein diet used for the animal feeding trials is given in Table 5.1. Cornstarch (Anil Starch, Ahmedabad, India) was used to dilute the protein content of the diets. The mineral mixture, vitaminized starch, and vitaminized oil were prepared as per ISI (1974) guidelines.

Table 5.1 Composition of experimental diets for PER, NPR, and nitrogen balance studies (gram per 100 g)

	Diets					
Ingredients	GEF-LC	GEF-RT	DEF-BP	PEF	SMP	Nonprotein
Enteral foods	67.5	71.4	60.2	96.0	–	–
Refined peanut oil	2.0	4.0	–	–	9.0	9.0
SMP	–	–	–	–	28.6	–
Corn starch	24.5	18.6	33.8	–	56.4	85.0
Vitaminized oil[a]	1.0	1.0	1.0	1.0	1.0	1.0
Vitaminized starch[a]	1.0	1.0	1.0	1.0	1.0	1.0
Salt mixture[a]	4.0	4.0	4.0	4.0	4.0	4.0

SMP skimmed milk powder taken as reference protein (RP)
[a]Prepared following ISI guidelines no. 7481 (1974)

Protein Efficiency Ratio

Male weanling albino rats (Wistar strain) weighing 38 ± 2 g were grouped into 10 animals per group by randomized block design and housed individually in wire mesh cages. Known amount of the test diet was wetted with warm water to a semi-solid consistency to prevent spillage. The animals had free access to ad libitum quantity of food and water. The food residue was collected, dried, and weighed to record the quantity of food consumed. The rats were weighed once a week and the experiment was confined to 4 weeks. The PER, as measured by the gain in body weight of the animal per gram of the protein consumed from the test food (Osborne et al., 1919), relative PER (RPER), and feed efficacy ratio (FER) were calculated as follows:

PER = gain in body weight (g)/protein intake (g)
RPER = (PER of test diet/PER of reference diet) x 100
FER = Weight gain (g)/food intake (g)

Net Protein Ratio

The NPR is the weight gain of a test animal plus weight loss of a control animal per gram of protein consumed. A nonprotein diet was fed to a group of 10 rats along with animals fed on PER experimental diets. At the end of 10 days, the changes in body weight and food intake by animals fed on nonprotein diet and experimental diets were noted. The NPR was calculated as per the method of Pellett and Young (1980).

NPR = {Weight gain of test animals (g) + average weight loss of animals fed on nonprotein diet (g)} /protein consumed by test animals (g)
Relativbe NPR = (NPR of test diet/NPR of reference diet) x 100

RPER and RNPR values were calculated according to the methodology suggested by Sarwar and Peace (1994).

Histopathology of Liver Tissue

At the end of the PER experiment, five rats selected randomly from each of the groups were sacrificed and the liver was dissected and separated. The fresh livers were weighed. A small portion of the liver tissue was fixed in 10% formalin for 24 h and subjected to dehydration using alcohol with graded strength and then infiltrated in paraffin wax. Thin sections (about 6-mm thickness) of the liver tissues were stained with hematoxylin and eosin, and viewed under the microscope for fatty infiltration (Raghuramulu et al., 1983). Moisture and fat contents of the livers were also estimated as per AACC (1983) methods. Besides the liver, kidney, heart, brain, spleen, pancreas, and lungs of the animals were also dissected, weighed, and examined visually as well as under microscope for deformation or any other unhealthy symptoms or morphological alterations.

Nitrogen Balance Studies

Male albino (Wistar Strain) rats, weighing 54 ± 4 g, were grouped into six rats in each group and housed in individual metabolic cages. The cages were fitted with steel funnels and perforated discs to facilitate collection of feces and urine separately. The experimental groups received enteral food diets containing 10% protein and another group received nonprotein diet. Carmine (0.2%) was used as a fecal marker at the beginning and at the end of the experimental period of 5 days (Bacon, 1980). Thymol crystals and a few drops of toluene were added as preservatives to the urine during collection to prevent fungal growth. Urine and feces were collected separately from individual rats for the experimental period and pooled. The urine was filtered using glass wool to free it from foreign materials. The feces were dried and weighed. Food residue was also dried and weighed. Nitrogen content of urine and feces was estimated by micro-Kjeldahl method, and the BV, TD, and NPU were calculated as per the method suggested by Pellett and Young (1980).

Biologocal value (*BV*): Biological value measures the proportion of absorbed nitrogen, which is retained and presumably utilized for protein synthesis, and therefore reflects true protein quality (Srikantia, 1981). It is the difference of nitrogen digested and nitrogen lost in metabolism divided by the nitrogen digested expressed in percentage.

$$BV = \{In - (Fn - Fe) - (Un - Ue)\} / \{In - (Fn - Fe)\} \times 100$$

True digestibility (*TD*): The true digestibility (TD) of a protein is the digestibility of an ingested protein expressed in percentage and is calculated as follows.

$$TD = \{In - (Fn - Fe)\} / In \times 100$$

Net protein utilization: NPU measures the difference in carcass nitrogen between rats fed on a test protein (NPU, carcass) and rats fed on a protein-free diet (Bender and Miller, 1953). It is calculated using the following formula:

$$NPU = (BV \times TD)/100$$

where In = nitrogen intake, Fn = fecal nitrogen of test animals, Fe = endogenous fecal nitrogen (fecal nitrogen of nonprotein group), Un = urinary nitrogen of test animal, Ue = endogenous urinary nitrogen (urinary nitrogen of nonprotein group).

Amino Acid Score/Rating

In 1946, Block and Mitchell introduced the concept of assessing the nutritional quality of a protein on the basis of its constituent amino acids. The value obtained was called chemical score, which is also known as amino acid score. The amino acid score and the amino acid rating of the enteral food proteins were calculated using the following formula:

Amino acid score = milligram of amino acid in one gram of test protein/milligram of amino acid in one gram of reference protein × 100

Amino acid rating = amino acid score × total protein (grams per 100 kcal)

Protein Digestibility Corrected Amino Acid Score (PDCAAS)

Amino acid scores corrected for true digestibility of protein (as determined by animal experimentation) are termed as protein digestibility-corrected amino acid scores. Based on the true digestibility and the amino acid content of the food protein, the PDCAAS was calculated as per FAO's (1991) methodology.

PDCAAS = Lowest amino acid ratio × true digestibility of protein

The data were analyzed by one-way ANOVA and Duncan's new multiple range test (Steel and Torrie, 1980).

Results and Discussion

The foods exhibited good growth-promoting qualities as reflected by the high PER values (Table 5.2). The PER of the low-cost general-cagory enteral food (GEF-LC) was 2.9, which was slightly higher than the ready-to-eat (GEF-RT) counterpart (2.6). This could be due to loss of available lysine during spray drying. On the other hand, the PER of disease-specific enteral food (DEF-BP) was significantly higher (3.4) than that of both the general-cagory foods, and was equivalent to the PER of reference protein (3.5) and of PEF (3.6). The higher PER value for the DEF-BP may be due to good quality protein of its ingredients, namely egg, milk, and lysine-rich grain amaranth. The weanling rats consumed higher quantity of the malt-based enteral foods than the PEF. This indicated the better palatability of the malt foods. The PER values of these malt-based foods are comparable with the earlier reports on PER values of foods based on malted cereals, legumes, and milk (Malleshi et al., 1989; Livingstone et al., 1993; Wondimu and Malleshi, 1996; Sumathi, 1997). However, NPR values for the enteral food formulae were lower than the PEF

Table 5.2 Protein efficiency ratio (PER), relative protein efficiency ratio (RPER), and feed efficiency ratio (FER) of the enteral food

Diets	Initial weight (g)	Food intake (g)	Protein intake (g)	Weight gain (g)	PER	RPER	FER
GEF-LC	37.0	257.7^{c}	24.6^{c}	72.0^{c}	2.9^{b}	83.9^{b}	0.28^{b}
GEF-RT	37.0	212.5^{a}	19.1^{a}	49.7^{a}	2.6^{a}	74.8^{a}	0.22^{a}
DEF-BP	37.0	286.2^{b}	30.3^{b}	102.3^{cd}	3.4^{c}	97.2^{c}	0.36^{b}
PEF	37.0	203.0^{a}	19.3^{a}	69.0^{b}	3.6^{c}	103.2^{d}	0.34^{c}
SMP	36.9	305.5^{b}	29.6^{b}	103.3^{cd}	3.5^{c}	–	0.33^{cd}
SEM	±4.1	±7.15	±0.69	±4.10	±0.09	±3.07	±0.008

Mean of the same column followed by different letters differs significantly ($p < 0.05$) according to Duncan's new multiple range test

and the reference protein (Table 5.3). The growth-promoting qualities of DEF-BP measured in terms of NPR and RNPR were significantly higher than that of GEF-LC and GEF-RT and were comparable to that of reference protein.

The fat content of the liver tissues of the rats fed with DEF-BP (16.4%) was higher than those fed with GEF-LC (11.4%) and GEF-RT (15.2%). However, there was no significant difference in the moisture content of the livers of rats fed with different diets including reference protein (Table 5.4).

The microscopic examination of the section of liver tissues of the animals fed with enteral food diets did not show any fatty infiltration, thus revealing that the proteins of the formulated enteral foods contained balanced amino acids (FAO, 1991). The examination of other vital organs also showed normal morphological pictures revealing that the foods were free from toxic constituents (Figs. 5.1 and 5.2).

The biological value of DEF-BP (94.0) was equal to the PEF (93.7). The BV of GEF-LC (88.9) was slightly higher than the BV of GEF-RT (85.8), and both were significantly lower than that of DEF-BP and the reference protein (Table 5.5). Similarly, the true digestibility and NPU values of DEF-BP were higher than in

Table 5.3 Net protein ratio (NPR) and relative net protein ratio (RNPR) of the enteral foods

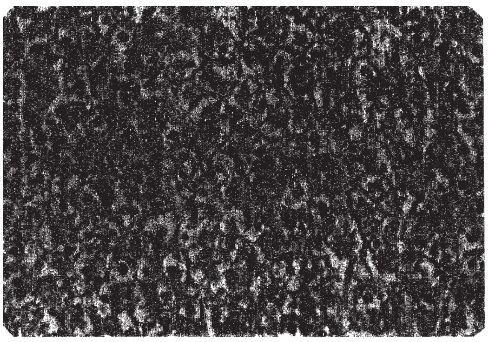
Diets	Initial weight (g)	Food intake (g)	Protein intake (g)	Weight gain (g) (after 10 days)	NPR	RNPR
Nonprotein	37.0	–	–	−6.8	–	–
GEF-LC	37.0	67.7^{a}	6.5^{a}	23.0^{b}	4.6^{b}	79.6^{a}
GEF-RT	37.0	53.7^{bc}	4.8^{b}	14.7^{c}	4.4^{b}	77.3^{a}
DEF-BP	37.0	56.9^{b}	6.0^{b}	25.8^{ab}	5.4^{a}	93.6^{b}
PEF	37.0	47.1^{c}	4.6^{b}	27.1^{ab}	7.5^{c}	130.8^{c}
SMP	36.9	68.1^{a}	6.6^{a}	31.3^{a}	5.7^{a}	–
SEM	±4.1	±2.4	±0.2	±1.8	±0.2	±3.9

Mean of the same column followed by different letters differs significantly ($p < 0.05$) according to Duncan's new multiple range test

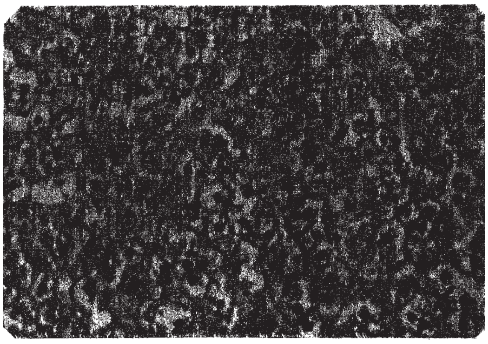
Table 5.4 Liver characteristics of rats fed with enteral food

Diet	Moisture content of liver (%)	Fat content of liver (%)	Histopathological examination
GEF-LC	68.6^{a}	11.4^{ab}	Normal, no fatty infiltration
GEF-RT	68.8	15.2^{cd}	Same
DEF-BP	66.2^{b}	16.4^{d}	Same
PEF	67.8^{ab}	13.5^{bc}	Same
SMP	69.0^{a}	10.1^{a}	Same
Sem (20df)	±0.60	±0.90	

Mean of the same column followed by different letters differs significantly ($p < 0.05$) according to Duncan's new multiple range test



GEF-LC



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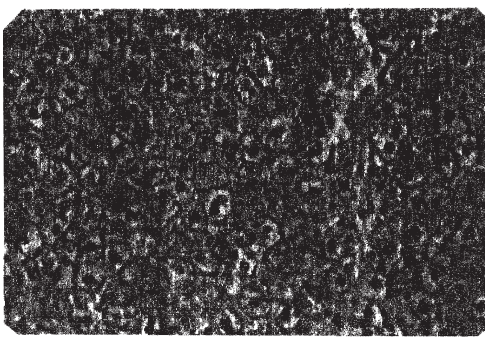
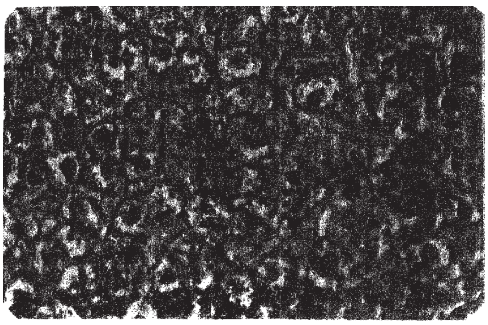


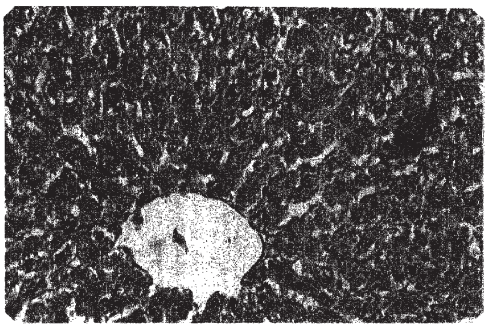
DEF-BP

Fig. 5.1 Photomicrographs of liver tissues of the rats fed with enteral food diets

general-cagory foods. The NPU values, which were over 75 for the enteral foods, indicated the good quality protein of the foods (Pellet and Young, 1980).

The protein sources of the enteral foods being derived from natural food ingredients were mostly of intact form. It has been reported that foods having intact protein are nutritionally as well as physiologically superior to foods having fractionated proteins such as peptides or amino acids (Silk, 1987).





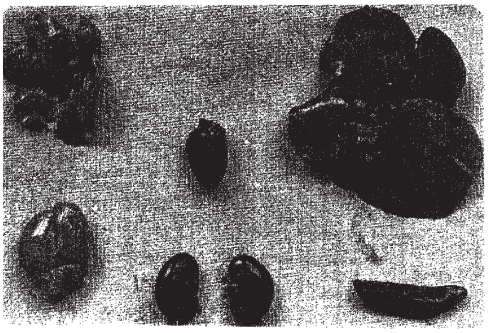


Fig. 5.2 Photomicrographs of liver tissues of the rats fed with PEF and reference protein and photographs of the vital organs from the rats fed on GEF-LC diet

The amino acid score and the PDCAAS of the enteral food proteins have been presented in Table 5.6. A slightly lower amino acid score for GEF-RT and DEF-BP as compared with that of GEF-LC could be due to the loss of lysine during spray drying. Although leucine was limiting in GEF-LC and lysine was limiting in GEF-RT and DEF-BP, the chemical scores of GEF-LC, GEF-RT, and DEF-BP

Table 5.5 Biological value (BV), true digestibility (TD), and net protein utilization (NPU) values for enteral foods

Diet	N intake* (mg)	N excreted*		N retained*	N balance* (retained)	TD	BV	NPU
		Fecal	Urinary					
GEF-LC	645.6[a]	78.0[a]	62.0[b]	505.5	77.8[b]	87.7[b]	88.9[cd]	78.0[b]
GEF-RT	618.0[a]	76.3[a]	78.0[c]	521.3	75.15[b]	87.6[b]	85.8[d]	75.2[b]
DEF-BP	802.0[a]	77.9[a]	43.3[abc]	680.8	84.0[a]	90.3[ab]	94.0[ab]	84.9[a]
PEF	458.0[a]	39.4[b]	26.8[a]	391.8[b]	85.5[a]	91.4[a]	93.7[ab]	84.0[a]
SMP	750.1[a]	62.4[ab]	24.6[a]	663.1	87.0[a]	91.6[a]	96.3[a]	88.3[a]
SEM (20 df)	±78.5	±9.2	±17.1	±120.1	±4.2	±1.1	±1.2	±1.6

Mean of the same column followed by different letters differs significantly ($p < 0.05$) according to Duncan's new multiple range test

*Values are average of 5 days total intake and excretion

Table 5.6 Essential amino acid contents, amino acid score, and protein digestibility corrected amino acid scores (PDCASS) of the enteral foods

Amino acid	EAA of the enteral foods (mg g^{-1} protein)			Suggested pattern of requirement for pre-school child[a]	Amino acid score[a] for enteral foods		
	GEF-LC	GEF-RT	DEF-BP		GEF-LC	GEF-RT	DEF-BP
Histidine	30.7	28.5	25.2	19.0	100.0	100.0	100.0
Isoleucine	28.8	24.3	27.3	28.0	100.0	87.0	98.0
Leucine	62.2	60.8	59.2	66.0	94.0	92.0	90.0
Lysine	61.1	47.3	50.4	58.0	100.0	82.0	87.0
Methionine + cystine	32.3	31.0	33.5	25.0	100.0	100.0	100.0
Phenylalanine + tyrosine	69.1	93.9	90.3	63.0	100.0	100.0	100.0
Threonine	42.3	39.6	42.0	34.0	100.0	100.0	100.0
Tryptophan	12.0	10.0	12.1	11.0	100.0	90.0	100.0
Valine	59.8	33.7	46.8	35.0	100.0	96.0	100.0
PDCAAS[a] (%)	–	–	–	–	82.0	72.0	79.0

[a]Amino acid score and the PDCAAS have been calculated by using FAO/WHO/UNU suggested pattern of amino acid requirements for preschool children. Values above 100 are taken as 100.

were 94, 82, and 87, respectively. The PDCAAS of GEF-LC (82%) was slightly higher than that of GEF-RT (72%) and DEF-BP (79%). Amino acid rating values for GEF-LC, GEF-RT, and DEF-BP were 366, 310, and 400, respectively. Lowry et al. (1990) reported lower amino acid score for some of the marketed enteral foods, and they attributed loss of lysine due to heat treatment during processing of the ingredients for the same. The summary of the results of protein quality evaluation presented in Fig. 5.3 reveals that the food proteins were of high biological

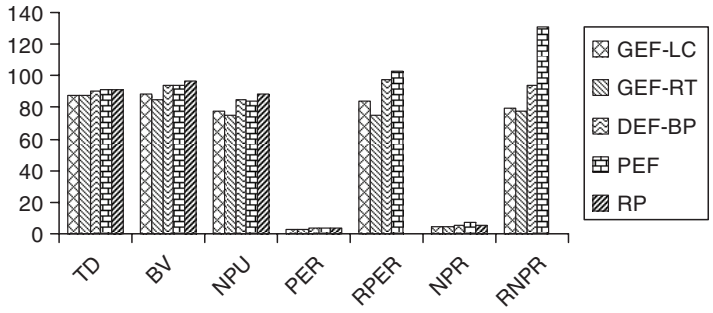


Fig. 5.3 Summary of the protein quality evaluation of the enteral food determined by animal feeding trials

value and also of good growth-promoting qualities. It can be expected that the foods will be suitable for patients for not only improving their nutritional status, but also in tissue building, which will be of paramount importance in many of the physiological conditions.

Baker and Han (1993) conducted rat bioassay to assess the adequacy of tryptophan and cystine of some enteral products and their influence on the protein quality when fresh and also on storage beyond shelf-life. They found that the cystine was the first limiting amino acid in both fresh and stored foods. They also observed that the PER of stored product was slightly lower than that of the fresh product and the controlled one. Sarwar and Peace (1994) studied the protein quality of six enteral foods of North America by animal feeding trials and reported that their true digestibility values ranged from 96 to 100. They also reported highest PER for diet containing 8% protein (casein diet) fortified with 0.2% l-methionine (control diet). Further, they reported that RPER values (42–56%) and the RNPR (67–74%) were markedly lower for the experimental products than for the control food (100%). As per Jones et al. (1983), the polymeric diets containing whole protein performed better than the chemically defined elemental diets, wherein free amino acids formed the nitrogen source, when administered to patients with normal gastrointestinal functions. It was shown by Gupta et al. (1958) that amino acids fed as intact protein to adult rats disappeared from the digestive tract into the body as rapidly as those fed in free form. Similar observation was also made by Silk (1987).

Trocki et al. (1986) also reported that in case of burned guinea pigs, the use of intact protein in enteral feeding as a nitrogen source could preserve body protein better than the use of free amino acids as a source of nitrogen. It was also reported by Young et al. (1978) that intact protein maintains mucosal mass better than crystalline amino acids.

Makola (2005) reviewed the superiority of elemental formulae containing free amino acids or hydrolyzed protein over polymeric formulae containing intact protein and concluded that polymeric formulae with intact protein were as effective as elemental formulae in providing nutritional support in majority of patients with gastrointestinal disorders.

More recently Lochs et al. (2006) developed an evidence-based guideline for indication, application, and types of formulae for enteral nutrition (oral or tube feeding) in patients with Crohn's disease, ulcerative colitis, and short bowel syndromes. This guideline was developed in accordance with officially accepted standards and was based on all relevant publications since 1985. They reported no significant differences in the effectiveness of free amino acids, peptide-based and whole (intact) protein formulae for the enteral nutritional support (tube feeding) of these patients. In addition, enteral nutritional support with formulae containing free amino acids is costlier than those formulae containing intact protein.

The results of the protein quality evaluation in the present study have also shown that GEF-LC, GEF-RT, and DEF-BP possess protein of high biological value although their proteins are in intact form.

Summary and Conclusion

The growth-promoting quality of the enteral foods was determined by rat feeding trials, and the protein quality was determined by nitrogen balance studies. The foods exhibited highly satisfactory growth-promoting qualities as measured in terms of the PER values, which were 2.9, 2.6, and 3.4 for GEF-LC, GEF-RT, and DEF-BP, respectively. The TD and BV of the foods ranged from 87.6 to 90.3 and from 85.8 to 94.0, respectively, thus indicating balanced amino acid profile of the food proteins. The vital organs of the rats fed with enteral foods were of normal architecture, thus revealing that the foods were free from toxic substances.

In conclusion, it can be stated that the enteral foods based on malted cereals, milk, and egg possess good growth-promoting qualities and their proteins are of high biological values. Therefore, it is possible to provide cost-effective and optimal nutritional support to patients through malt-based enteral foods.

Chapter 6
Shelf-Life Studies and Sensory Evaluation of Enteral Foods

Introduction

This chapter enumerates the shelf-life and sensory evaluation studies conducted on these enteral foods. Shelf-life is an important property of a food that interests everyone in the food chain – from manufacturer to consumer. Because shelf-life of a food product is associated with consumer safety and satisfaction, precisely evaluating the shelf-life of a medical food is important to optimize patients' safety, satisfaction, and clinical outcome. Information relating to shelf-life is also critical to the purchasing agent in both the retail and food service trade. Measuring the sensory properties and determining the importance of these properties are also important aspects of any new food development program including medical foods. Also, because sensory perception and food acceptance is strongly correlated, sensory evaluation can help predict the acceptance of the medical food by the patients. The taste, aroma, and texture mainly govern the acceptance or enjoyment of a food including medical food. This is particularly important when a patient receives the medical food orally. The question arises: what is the significance of taste, aroma, and texture of medical food when it is delivered via tube? The answer is that appropriate measurement and evaluation of sensory principles govern not only patients' food acceptance, but also predict stability and integrity of the food, and thereby establish the food safety. Studies have shown that physicochemical analysis of medical food alone does not provide sufficient data to allow prediction of either patient acceptance or product integrity (Thomas et al., 1981; Winger, 2000). Hence, sensory evaluation of medical food is a critical tool in the assessment of product stability and consumer acceptance, and thus should be considered as an integral part of a medical food stability program. Because changes in sensory attributes of a food are related to shelf-life of the food, the shelf-life and sensory evaluation studies conducted on the three medical foods developed in the present study are discussed here.

M. Kaur, *Medical Foods from Natural Sources,*
DOI: 10.1007/978-0-387-79378-8_6,

Materials and Methods

Shelf-Life

Shelf-life of a food is the period of time determined from the harvest or production of the food till the food is perceived as significantly different in quality from the fresh one in terms of physicochemical, nutritional, microbiological, and sensory properties, among others. Hence, to evaluate shelf-life of a food, it is important to analyze these properties using the standard procedures. The methodologies followed to evaluate the shelf-life of the food are as follows.

Sorption Studies

The knowledge of sorption isotherm behavior of a food helps predict stability and quality changes during packaging and storage. It also helps to choose an appropriate packaging material to enhance the shelf-life of a food or any material. The relationship between water content and water activity of a food is complex. An increase in water activity is usually accompanied by an increase in water content but in a nonlinear fashion. At equilibrium, the relationship between water content and equilibrium humidity of a food can be displayed graphically by a curve, which is known as sorption isotherm. For each humidity value, a sorption isotherm indicates the corresponding water content value at a given constant temperature. If the composition or quality of the food changes, then its sorption behavior also changes. Because of the complexity of sorption processes, the isotherms cannot be determined by calculation, but must be recorded experimentally for each product. Therefore, sorption studies on the enteral foods were conducted.

The sorption isotherms of the enteral foods were carried out at 27°C. Samples (8–10 g) in duplicate were exposed to atmospheres of 11–92% relative humidity (RH) built up in desiccators using saturated salt solutions (Table 6.1) as per the methodology

Table 6.1 Saturated salt solutions and their relative humidities (RH)

Saturated Salts	RH (%)
Lithium chloride (LiCl)	11
Potassium acetate (CH_3COOK)	22
Magnesium chloride ($MgCl_2$)	32
Potassium carbonate (K_2CO_3)	44
Sodium bromide (NaBr)	56
Sodium nitrite ($NaNO_2$)	64
Sodium chloride (NaCl)	75
Potassium chromate (K_2CrO_4)	86
Potassium nitrate (KNO_3)	92

suggested by Rockland (1960). The samples were weighed periodically and retained in the respective desiccators till they attained constant weight (equilibrium moisture levels), or the onset of mold growth whichever occurred earlier.

Packaging and Storage

Details about the packaging and storage studies are as follows.

Packaging materials and storage condition

The sorption isotherm studies revealed that the GEF-LC was less hygroscopic, whereas the GEF-RT and DEF-BP were moderately hygroscopic. Hence, low-cost flexible packaging material – polypropylene (pp) – was used for packaging the GEF-LC, whereas the other two foods were packed in foil laminate pouches. One-hundred gram sample of each of the foods was packed in unit pouches (15 cm × 20 cm) made from three kinds of flexible packaging materials. The packaging materials used and their water vapor transmission rates (WVTR), oxygen transmission rate (OTR), packaging and storage conditions, and the quality criteria evaluated during storage are presented in Table 6.2.

The samples were withdrawn periodically and analyzed for changes in moisture content, free fatty acids (FFA), peroxide value (PV), and changes in pH following the method of AOAC (1980).

Sensory Quality Evaluation

Sensory quality evaluations on the foods were carried out based on a score card developed for a set of attributes such as appearance, flavor, taste, consistency, doneness, and overall acceptability. On each withdrawal, the samples were reconstituted in warm water (the GEF-LC was cooked) and evaluated for the sensory attributes (Table 6.3) by a panel of 10 members who were trained for sensory evaluation. The taste panel was also requested to identify the flavor of the foods and to write a remark on the sensory quality of the foods.

Microbiological Safety

The microbiological safety evaluation of the fresh and stored foods was carried out according to the procedure suggested by Vanderzant and Splittstoesser (1992). The details of this procedure are discussed in Chap. 4. The microbiological safety of fresh enteral foods is presented in Chap 4. In this chapter, the microbial safety of the food evaluated at the beginning, midcourse, and at the terminal stages of storage studies is discussed.

Table 6.2 Details of shelf-life studies of the enteral foods

A: Packaging materials (specifications)

Packaging materials		WVTR (g m^{-2} day^{-1} under 90% RH gradients at 38°C)	OTR (cc m^{-2} day^{-1} under atmosphere at 27°C)
PP	Polypropylene (150 gauge)	4.0	1,800–2,000
MP	12-micron metallized polyester/150-gauge polyethylene	1.8	30–40
AF	12-micron polyester/12-micron aluminum foil/150-gauge polyethylene	Nil	Nil

B: Storage condition

Ambient: (27 ± 2)°C and (65 ± 2)% RH	Accelerated: (38 ± 2)°C and (92 ± 2)% RH

C: Packaging and storage particulars of enteral foods

Foods	Packaging material	Packaging condition	Storage condition
GEF-LC	PP MP AF	Air	Ambient and accelerated
GEF-RT	MP AF	Air	Same
DEF-BP	MP AF	Air	Same
DEF-BP	MP AF	Vacuum	Same

D: Quality criteria of evaluation of foods on each withdrawal

a. Physical	Color, appearance, consistency of the slurry
b. Chemical	Moisture, FFA, PV, pH
c. Sensory	Appearance, flavor, taste, consistency, overall acceptability
d. Microbiological safety	Mesophilic aerobes, lactics, yeasts and molds, mesophilic spore formers

E: Statistical analyses

Statistical analyses of sensory attributes by Duncan's new multiple range test

WVTR water vapor transmission rate, *OTR* oxygen transmission rate, *PP* polypropylene, *MP* metallized polyester, *AF* aluminum foil, *FFA* free fatty acids, *PV* peroxide value, *RH* relative humidity

Results and Discussion

Sorption Studies

The sorption behavior of the low-cost general-cagory enteral food (GEF-LC) was typical of cereal-legume blend. Although the food contained about 20% whole milk powder, it did not exhibit high hygroscopicity. The food retained its freshness and free-flowing properties at 60% relative humidity (RH), and absorbed moisture at higher RH followed by becoming soggy and lumpy, and subsequently developed mold growth at 86% moisture after 2 weeks of exposure (Table 6.4). Based on these observations, it could be inferred that the critical moisture and humidity for the safe

Table 6.3 Score card for the sensory evaluation of enteral foods

Appearance (Color)

White	Cream	Brown

Flavor

Pleasant	Moderate	Unpleasant

Taste

Bland	Sweet	Very sweet

Consistency

Watery with sediments	Uniform	Lumpy

Doneness

Semi-cooked	Cooked	Burnt

Overall acceptability

Unacceptable	Acceptable	Highly Acceptable

Please identify and record the flavor:_____

Remarks:_____

Date:_____

storage of the GEF-LC could be about 8% moisture and 60% RH, respectively (Fig. 6.1). In order to protect these types of products from being spoiled by mold growth during storage, the use of moisture-proof flexible packing materials such as high-density polypropylene (HDPP) and high-density polyethylene (HDPE) pouches may be considered (Bhaskaran et al., 2000). On the other hand, sorption behavior of the spray-dried enteral foods such as general-cagory ready-to-eat (GEF-RT) and disease-specific (DEF-BP) enteral foods was intermediate to spray-dried milk powder and cereal-legume blends (Malleshi et al., 1989). Out of the two spray-dried foods, the hygroscopicity of GEF-RT was slightly higher than that of DEF-BP. Both the foods retained their freshness and free-flowing properties up to 56% RH, after which lumping and caking occurred. The onset of fungal growth was noticed in GEF-RT after 2 weeks of exposure at 92% RH, whereas the fungal growth was observed at 3 weeks in case of DEF-BP at 92% RH. The critical moisture and humidity for safe storage for both the spray-dried foods were 6% moisture and 44% RH (Fig. 6.1).

Table 6.4 Moisture-sorption relationship of the enteral foods at 27°C

	Enteral foods					
	GEF-LC		GEF-RT		DEF-BP	
Relative humidity (%)	EMC (dwb)	Observations	EMC (dwb)	Observations	EMC (dwb)	Observations
11	4.6	Color and flavor retained, free-flowing	2.8	Color and flavor retained, free-flowing	2.8	Color and flavor retained, free-flowing
22	5.2	Same as above	3.4	Same as above	3.4	Same as above
32	6.5	Same as above	5.3	Same as above	4.6	Same as above
44	8.0	Same as above	6.2	Same as above	5.8	Same as above
56	9.7	Same as above	9.4	Same as above	8.0	Same as above
64	10.4	Free-flowing	12.8	Slightly soggy	11.2	Same as above
75	13.6	Color slightly dull, rancid flavor, free-flowing	16.0	Color slightly dull; caking starts after 3 weeks	14.6	Color acceptable, slightly soggy
86	20.6	Color dull, rancid flavor, slightly lumpy, visible mold growth after 3 weeks	24.0	Color dull, slight off flavor, visible mold growth after 18 days, caked	21.7	Color slightly dull, soft texture, visible mold growth after 23 days
92	22.4	Color dull, rancid flavor, caked, visible mold growth after 2 weeks	25.8	Color dull, off flavor, caked, visible mold growth after 2 weeks	23.5	Color dull flavor reversion, visible mold growth after 18 days

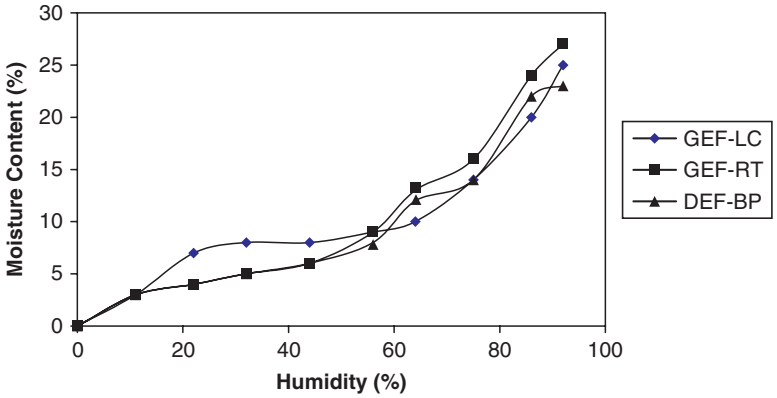


Fig. 6.1 Sorption isotherm of enteral foods

These observations revealed that these foods needed to be packed in 100% moisture barrier packaging materials such as aluminum foil laminate (AF), glass bottles, or metallic tins. However, for a short storage period, these foods could be packed in HDPE pouches.

Packaging and Stroage

The changes in moisture, FFA, and PV during storage of the GEF-LC packed in PP, MP, and AF and stored at ambient and accelerated storage conditions are presented in Fig. 6.2a-f. The changes in moisture and FFA content of GEF-LC were in line with the nature of the packaging material as well as storage conditions. As expected, there was no change in the moisture content of the food packed in AF. However, moisture content of the food increased from the initial level of 5.6 to 6.8 and 7.6% for the samples packed in MP and PP, respectively, on 180 days of storage at ambient storage condition. The GEF-LC sample packed in PP and MP and stored at accelerated storage condition contained 10.4 and 9.3% moisture, respectively, on 120 days of storage. Similar to moisture, the increase in FFA was also considerably higher

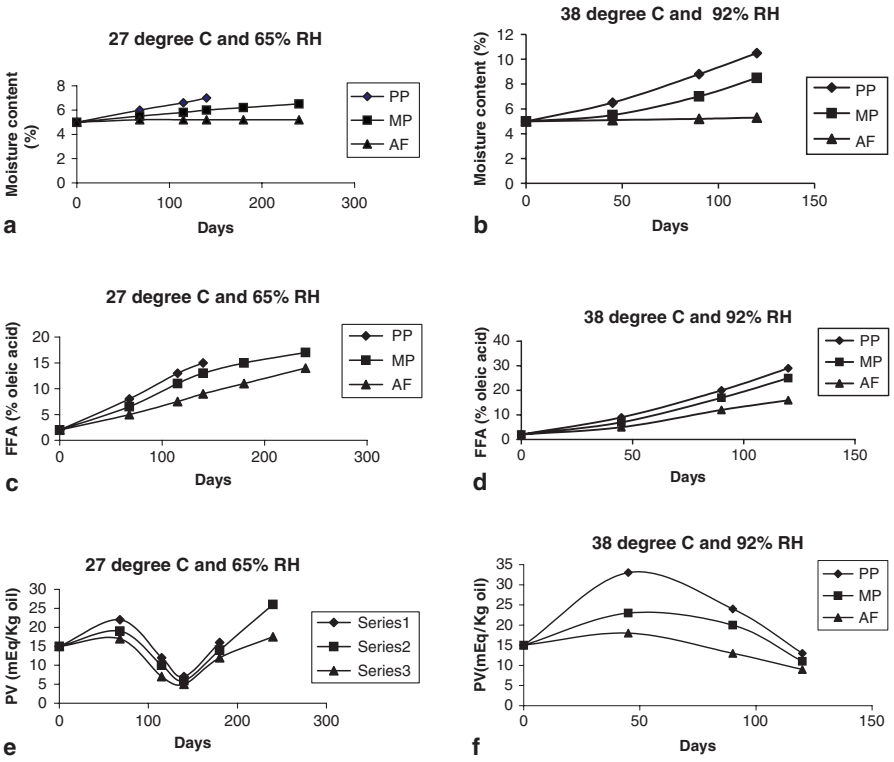


Fig. 6.2 Changes in moisture, FFA, and PV contents of GEF-LC during storage at ambient and accelerated storage conditions

for samples packed in PP than those packed in MP and AF in both the storage conditions. The increase in FFA was rapid in accelerated condition as compared with ambient condition.

The FFA content of the sample increased from the initial value of 3 to 30% in PP after 120 days of storage at accelerated storage condition, whereas the corresponding values for samples packed in MP and AF were 25 and 15%, respectively. Because the food contained malted cereals having active lipase in it, the lipolysis of fat from the cereals itself as well as from that of added fat could have taken place during storage resulting in considerable increase in FFA content. The changes in PV during storage were not consistent. The PV of the samples packed in PP pouches increased to about 25 units from initial value of 15 units after 60 days of storage, then dropped to five units after 140 days of storage, and again increased to about 30 units after 240 days of storage at ambient storage condition. On the other hand, in accelerated storage condition there was a steep rise in PV for the samples packed in PP pouches after 60 days (38 units), which dropped to its initial value (15 units) on 120 days of storage. However, the PV contents of the samples packed in MP and AF were lower than those packed in PP at all comparable time periods and storage conditions. Changes in pH of the food cooked in water after each withdrawal were also recorded. It was observed that the pH dropped slightly during storage (from 6.5 to 5.9). The pH dropped rapidly in samples stored at accelerated storage conditions.

The storage characteristics of the spray-dried enteral foods, namely the GEF-RT and DEF-BP are presented in Figs. 6.3a-f and 6.4a-f, respectively. Both the foods

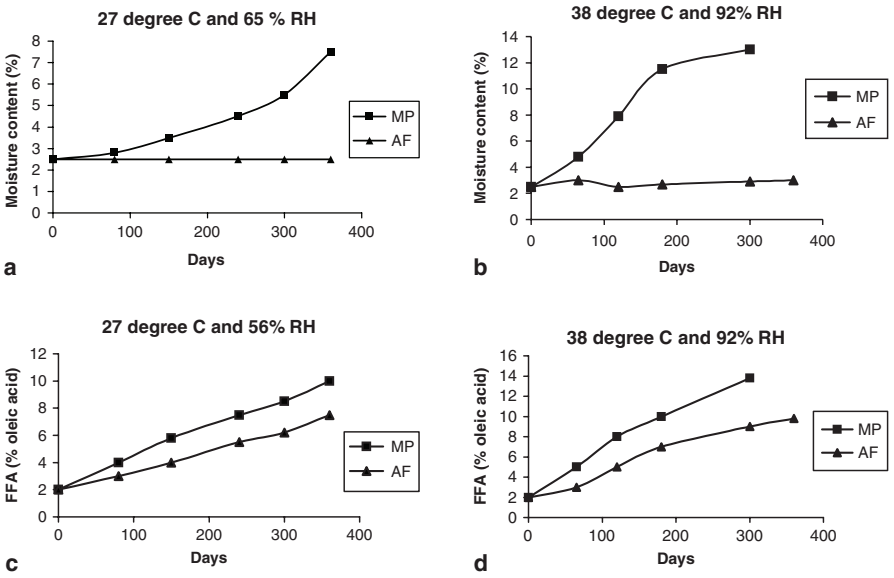


Fig. 6.3 Changes in moisture, FFA, and PV contents of GEF-RT during storage at ambient and accelerated storage conditions

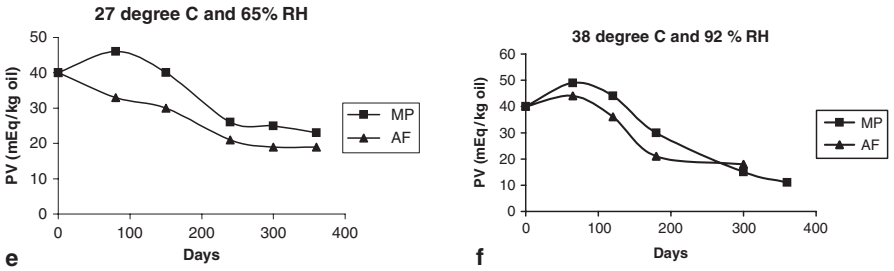


Fig. 6.3 (continued)

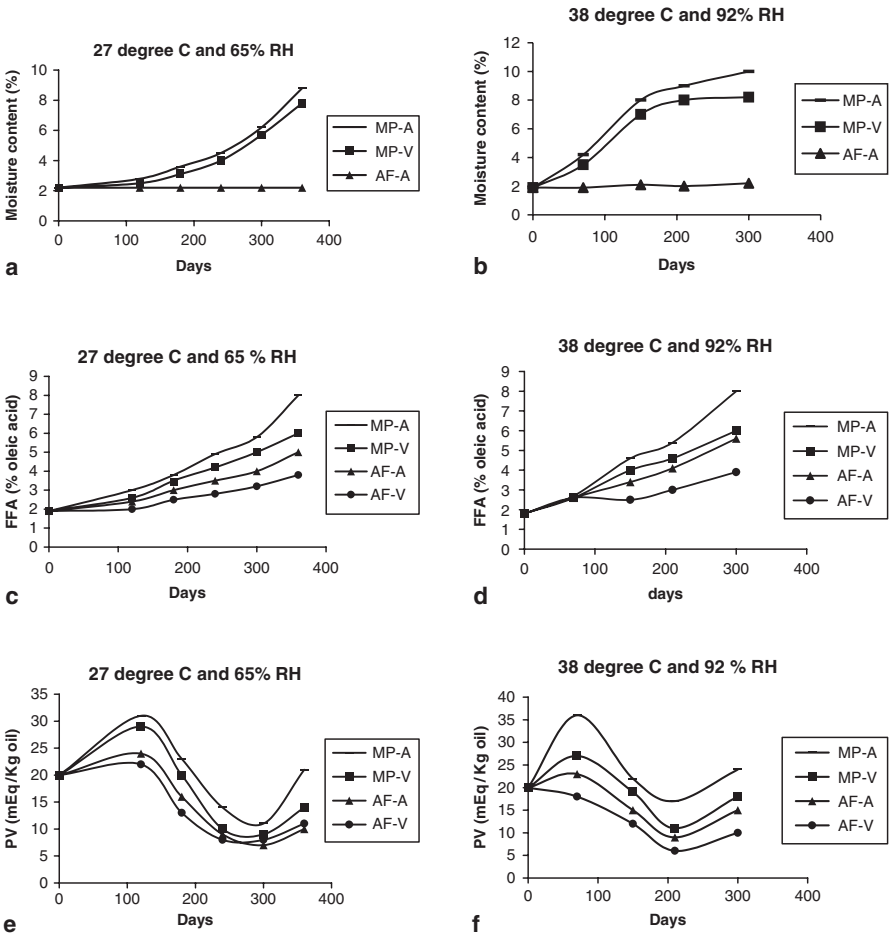


Fig. 6.4 Changes in moisture, FFA, and PV contents of DEF-BP during storage at ambient and accelerated storage conditions

showed similar trend with respect to changes in moisture, FFA, PV, and pH to that of GEF-LC. The samples packed in MP pouches absorbed moisture at both the storage conditions whereas there were negligible changes in moisture contents of the samples packed in AF laminates. However, increase in the moisture content of the samples stored at ambient storage condition was gradual, and the moisture content on 360 days of storage was only 8.2% for GEF-RT and 9.1% for DEF-BP, which was slightly higher than their critical moisture levels (6%) for safe storage. A rapid increase in moisture content was observed up to 120 days of storage for both the spray-dried foods stored in accelerated storage conditions, after which it was gradual. Likewise, the increase in FFA content was also gradual and the foods packed in MP pouches had higher FFA (9.2%) than those packed in AF laminate pouches (6.4%). The increase in FFA content was slightly lower for the vacuum-packed samples than the air-packed samples.

There was a considerable fluctuation in the PV content of GEF-RT and DEF-BP packed in MP and AF and stored at ambient as well as accelerated storage conditions. While there was a decrease in PV on a few days of storage for samples packed in AF laminates, it increased considerably in all the samples including the air- and the vacuum-packed samples packed in MP pouches. The pH of both the foods decreased slightly (from 6.0 to 5.7) on storage in all the packaging materials stored at both the storage conditions.

Sensory Qualities

The scores for various sensory attributes and the test of significance of GEF-LC packed in different packaging materials and stored at ambient and accelerated storage conditions were assessed on each withdrawal, and the scores are presented in Tables 6.5 and 6.6, respectively. The food packed in AF laminates and stored at refrigerated conditions was used as control and the same was evaluated for various sensory attributes. The test of significance was also performed on each withdrawal.

The appearance (color) of the samples was measured at the termination of the storage studies and was expressed as % reflectance against barium sulfate taken as standard (100% reflectance). The color of the foods turned slightly brown on storage, and the intensity of browning was the highest in samples packed in PP followed by MP and AF under both the storage conditions. The reflectance values for fresh samples of GEF-LC, GEF-RT, and DEF-BP were 50, 45, and 42% and of their counter parts at the terminal stages of storage packed in MP and stored at ambient condition were 45, 31, and 27, respectively.

The sensory scores for flavor and taste were higher for all the samples packed in AF than samples packed in PP and MP pouches, particularly for those samples stored at ambient storage conditions.

The observations on the sensory attributes for GEF-RT (Tables 6.7 and 6.8) and DEF-BP (Tables 6.9 and 6.10) were more or less similar. The consistency of all the

Table 6.5 Sensory attributes of GEF-LC stored at ambient and accelerated storage conditions

Attribute	Appearance		Flavor		Taste		Consistency		Doneness		Overall acceptability	
Storage condition	Amb	Acc	Amb	Acc	Amb	Acc	Amb	Acc	Amb	Acc	Amb	Acc
Storage period (days)	65	45	65	45	65	45	65	45	65	45	65	45
Control	4.9	4.4	3.5	4.4	4.0	3.7	4.9	4.9	4.7	4.7	3.5[a]	4.4[a]
PP	4.4	4.1	2.9	2.6	3.7	3.0	4.9	4.9	4.2	4.1	2.5[c]	2.5[c]
MP	4.5	4.1	3.0	2.8	3.8	3.0	4.9	4.9	4.5	4.1	2.9[b]	3.3[b]
AF	4.5	4.1	3.2	2.9	3.9	3.5	4.9	4.9	4.5	4.2	3.1[b]	3.6[b]
SEM (32 df)	±0.18	±0.21	±0.32	±0.20	±0.30	±0.30	±0.1	±0.11	±0.26	±0.38	±0.13	±0.23
Storage period (days)	115	90	115	90	115	90	115	90	115	90	115	90
Control	4.7	4.7	3.1	3.4	3.8	4.7	5.0	4.9	4.8	4.6	3.8[a]	3.8[a]
PP	4.3	2.9	2.3	2.7	2.9	4.4	4.9	4.8	4.2	4.1	2.6[b]	2.6[b]
MP	4.3	3.0	2.9	2.8	3.9	4.5	4.9	4.9	4.4	4.1	2.9[b]	2.9[b]
AF	4.5	4.3	3.0	3.3	3.8	4.6	4.9	4.9	4.6	4.4	3.1[b]	3.0[b]
SEM (32 df)	±0.23	±0.25	±0.28	±0.44	±0.32	±0.31	±0.1	±0.11	±0.47	±0.36	±0.19	±0.16
Storage period (days)	149	120	149	120	149	120	149	120	149	120	149	120
Control	4.4[a]	4.4[a]	2.7	2.9	3.8	3.7[a]	5.0	5.0	5.0	4.1	3.5	3.5
PP	2.4[b]	1.6[b]	2.1	2.2	2.7	2.2[b]	4.9	4.9	4.1	3.9	2.1	2.0
MP	2.7[b]	1.4[c]	2.4	2.4	3.7	2.0[b]	4.9	4.8	4.6	3.9	2.4	2.0
AF	4.3[a]	3.0[b]	2.6	2.6	3.7	3.6[a]	4.9	5.0	5.0	4.1	2.6	2.9
SEM (32 df)	±0.28	±0.24	±0.15	±0.21	±0.32	±0.42	±0.1	±0.10	±0.03	±0.10	±0.19	±0.21
Storage period (days)	210	150	210	150	210	150	210	150	210	150	210	150
Control	4.9[a]	4.3[a]	2.5	2.4	3.0	3.0	5.0	5.0	5.0	4.0	3.0	3.0
PP	2.3[b]	1.4[c]	2.0	2.0	1.5	1.4	4.9	4.8	4.0	3.0	1.8	1.6
MP	*	*	*	*	*	*	*	*	*	*	*	*
AF	*	2.8[b]	2.3	2.2	2.5	2.5	4.9	4.8	5.0	4.0	2.0	2.0
SEM (32 df)	±0.20	±0.20	±0.20	±0.20	±0.30	±0.40	±0.1	±0.10	±0.10	±0.10	±0.20	±0.20

Means of the same column followed by different letters differ significantly ($p < 0.05$) according to Duncan's new multiple range test
The same notations/ abbreviations used in Table 6.5 are used in Tables 6.6–6.10
Amb ambient [(27 ± 20)°C; (65 ± 2)% RH)], *ACC* accelerated [(38 ± 2)°C; (92 ± 2)% RH], *PP* polypropylene, *MP* metallized polyester/polyethylene, *AF* aluminum foil laminate
*Signifies that experiment is terminated

Table 6.6 Test of significance between storage period under different packaging conditions of GEF-LC

Storage period (days)	Amb (27°C and 65% RH)				Storage period (days)	Acc (38°C and 92% RH)			
	Control	PP	MP	AF		Control	PP	MP	AF
	Appearance					Appearance			
65	4.7	4.5	4.5[a]	4.9	45	4.7	4.1[a]	4.1	4.3[a]
115	4.5	4.3	4.3[a]	4.5	90	4.4	3.0	2.9[b]	4.3[a]
149	4.4	2.4	2.7[b]	4.3	120	4.4	1.8	1.4[c]	3.0[b]
210	4.0	*	*	3.0	150	4.2	*	*	2.8
SEM	±0.21 (32 df)	±0.27 (24 df)	±0.25 (24 df)	±0.18 (32 df)	SEM	±0.23 (32 df)	±0.25 (24 df)	±0.24 (24 df)	±0.21 (32 df)
	Flavor					Flavor			
65	3.5	3.0	3.2[a]	3.2[a]	45	3.5	2.6	2.7	3.3[a]
115	3.2	2.9	2.3[b]	3.1[ab]	90	2.8	2.2	2.3	3.0[ab]
149	3.0	2.4	2.1[b]	2.7[a]	120	2.9	2.0	2.1	2.4[bc]
210	3.0	*	*	2.1[c]	150	2.9	*	*	1.8[c]
SEM	±0.18 (32 df)	±0.27 (24 df)	±0.28 (24 df)	±0.13 (32 df)	SEM	±0.22 (32 df)	±0.24 (24 df)	±0.26 (24 df)	±0.29 (32 df)
	Taste					Taste			
65	4.0	3.8	3.8[a]	4.5	45	4.1	3.0	3.0[a]	3.5
115	3.7	3.8	2.9[b]	3.8	90	3.7	2.8	2.7[b]	3.5
149	3.7	3.2	2.7[b]	3.6	120	3.5	2.2	1.9[c]	3.3
210	3.6	*	*	3.0	150	3.6	*	*	3.2
SEM	±0.30 (32 df)	±0.34 (24 df)	±0.27 (24 df)	±0.27 (32 df)	SEM	±0.32 (32 df)	±0.41 (24 df)	±0.41 (24 df)	±0.41 (32 df)
	Consistency					Consistency			
65	5.0	4.8	4.8	4.8	45	5.0	4.9	4.9	4.9
115	4.4	4.8	4.8	4.6	90	4.9	4.9	4.8	4.9
149	4.4	4.8	4.6	4.6	120	4.7	4.7	4.5	4.5
210	4.2	*	*	4.6	150	4.7	*	*	4.0
SEM	±0.16 (32 df)	±0.12 (24 df)	±0.12 (24 df)	±0.18 (32 df)	SEM	±0.15 (32 df)	±0.13 (24 df)	±0.16 (24 df)	±0.21 (32 df)
	Doneness					Doneness			
65	4.7	4.6	4.7	45	4.7	4.1	4.1	4.4	

115	4.6	4.4	4.5	4.5	90	4.6	4.1	4.1	4.2
149	4.1	4.2	4.2	4.4	120	4.1	3.4	4.0	4.2
210	4.0	*	*	4.2	150	4.0	*	*	4.1
SEM	±0.15 (32 df)	±0.34 (24 df)	±0.31 (24 df)	±0.35 (32 df)	SEM	±0.31 (32 df)	±0.41 (24 df)	±0.43 (24 df)	±0.41 (32 df)
	Overall acceptability					Overall acceptability			
65	3.5	3.1[a]	3.0	3.5[a]	45	3.5	3.3[a]	3.6[a]	3.5[a]
115	3.4	3.0[ab]	2.9	3.2[b]	90	3.4	2.6[ab]	2.6[b]	3.0[b]
149	3.5	2.7[b]	2.8	3.0[b]	120	3.3	2.0[b]	2.0[c]	3.0[bc]
210	3.0	*	*	2.9[a]	150	3.3	*	*	1.7
SEM	±0.16 (32 df)	±0.32 (24 df)	±0.16 (24 df)	±0.18 (32 df)	SEM	±0.16 (32 df)	±0.26 (24 df)	±0.19 (24 df)	±0.22 (32 df)

Table 6.7 Sensory attributes of GEF-RT stored at ambient and accelerated storage conditions

Attribute	Appearance		Flavor		Taste		Consistency		Doneness		Overall acceptability	
Storage condition	Amb	Acc	Amb	Acc	Amb	Acc	Amb	Acc	Amb	Acc	Amb	Acc
Storage period (days)	80	65	80	65	80	65	80	65	80	65	80	65
Control	4.9	4.8	3.7	3.8	4.2	4.2	4.7	4.7	4.9	4.7	4.7	4.2
MP	4.5	4.6	3.4	3.3	3.9	3.7	4.7	4.1	4.6	4.2	4.3	4.3
AF	4.6	4.8	3.5	3.6	4.0	4.0	4.7	4.7	4.9	4.3	4.7	4.6
SEM (24 df)	±0.16	±0.23	±0.27	±0.35	±0.33	±0.34	±0.22	±0.33	±0.15	±0.41	±0.31	±0.35
Storage period (days)	150	120	150	120	150	120	150	120	150	120	150	120
Control	4.7	4.0	3.6	3.6	4.1	4.1	4.7	4.6	4.8	4.8	4.4	4.4
MP	4.0	3.3	3.0	3.0	3.7	3.7	4.6	4.6	4.6	4.7	4.0	4.0
AF	4.6	3.7	3.5	3.2	4.0	3.8	4.7	4.6	4.7	4.7	4.2	4.0
SEM (24 df)	±0.28	±0.36	±0.19	±0.27	±0.45	±0.37	±0.29	±0.33	±0.20	±0.27	±0.32	±0.24
Storage period (days)	240	180	240	180	240	180	240	180	240	180	240	180
Control	4.3	4.3	3.6	3.6	3.9	3.9	4.6	4.4	4.6	4.6	4.1	4.1
MP	3.8	*	3.0	*	3.4	*	4.4	*	4.5	*	3.6	*
AF	4.2	4.2	3.0	3.2	3.8	3.5	4.2	4.4	4.5	4.6	3.6	3.6
SEM	±0.35 (24 df)	±0.29 (16 df)	±0.24 (24 df)	±0.24 (16 df)	±0.33 (24 df)	±0.44 (16 df)	±0.33 (24 df)	±0.34 (16 df)	±0.21 (24 df)	±0.17 (16 df)	±0.35 (24 df)	±0.29 (16 df)
Storage period (days)	300	240	300	240	300	240	300	240	300	240	300	240
Control	4.2	4.2	3.4	3.4	3.2	3.5	4.4	4.4	4.6	4.5	3.9	3.9
MP	*	*	*	*	*	*	*	*	*	*	*	*
AF	3.6	4.2	2.5	3.0	2.7	3.2	4.4	4.3	4.5	4.4	3.4	3.4
SEM (16 df)	±0.28	±0.17	±0.20	±0.23	±0.27	±0.34	±0.16	±0.20	±0.33	±0.39	±0.24	±0.22

Means of the same column followed by different letters differ significantly ($p < 0.05$) according to Duncan's new multiple range testThe same notations/abbreviations are used in subsequent tables on shelf-life studies*Amb* ambient [(27 ± 2)°C; (65 ± 2)% RH], *ACC* accelerated [(38 ± 2)°C; (92 ± 2)% RH], *PP* polypropylene, *MP* metallized polyester/polyethylene, *AF* aluminum foil laminate

*Signifies that experiment is terminated

Table 6.8 Test of significance between storage condition under different packaging conditions of GEF-RT

Storage period (days)	Amb (27°C and 65% RH)			Storage period (days)	Acc (38°C and 92% RH)		
	Control	MP	AF		Control	MP	AF
	Appearance				Appearance		
80	4.8	4.6	4.9[a]	65	4.8	4.6[a]	4.7[a]
150	4.6	4.0	4.7[ab]	120	4.3	3.3[b]	4.7[ab]
240	4.4	3.8	4.2[abc]	180	4.2	*	4.1[ab]
300	4.4	*	3.7[c]	240	4.0	*	3.7[b]
SEM	±0.28 (32 df)	±0.29 (24 df)	±0.27 (32 df)		±0.28 (32 df)	±0.31 (16 df)	±0.25 (32 df)
	Flavor					Flavor	
80	3.7	3.4	3.6[a]	65	3.7	3.2	3.6
150	3.6	3.1	3.5[ab]	120	3.6	3.0	3.2
240	3.6	3.1	3.0[abc]	180	3.5	*	3.2
300	3.5	*	2.5[c]	240	3.4	*	3.2
SEM	±0.27 (32 df)	±0.22 (24 df)	±0.22		±0.27 (32 df)	±0.30 (16 df)	±0.29 (32 df)
	Taste				Taste		
80	4.2	3.9	4.3[a]	65	4.2	3.7	4.1
150	4.1	3.7	4.0[ab]	120	4.1	3.7	3.8
240	4.0	3.4	3.8[ab]	180	4.0	*	3.5
300	3.8	*	3.7[c]	240	3.8	*	3.5
SEM	±0.32 (32 df)	±0.39 (24 df)	±0.35 (32 df)		±0.34 (32 df)	±0.40 (16 df)	±0.38 (32 df)
	Consistency				Consistency		
80	4.7	4.7	4.7	65	4.7	4.6	4.7
150	4.6	4.7	4.7	120	4.6	4.1	4.6
240	4.4	4.2	4.5	180	4.4	*	4.4
300	4.4	*	4.5	240	4.3	*	4.3
SEM	±0.30 (24 df)	±0.36 (24 df)	±0.119 (32 df)		±0.30 (32 df)	±0.36 (16 df)	±0.28 (32 df)
	Doneness				Doneness		
80	4.8	4.6	4.9	65	4.8	4.7	4.7
150	4.7	4.3	4.5	120	4.7	4.2	4.6
240	4.6	3.5	4.5	180	4.6	*	4.4
300	4.5	*	4.5	240	4.4	*	4.3
SEM	±0.18 (24 df)	±0.35 (24 df)	±0.119 (32 df)		±0.27 (32 df)	±0.29 (16 df)	±0.31 (32 df)
	Overall acceptability				Overall acceptability		
80	4.7	4.3	4.4[a]	65	4.7	4.3	4.6[a]
150	4.4	4.3	4.3[ab]	120	4.4	4.1	4.2[ab]
240	4.1	3.5	3.8[ab]	180	4.1	*	3.6[b]
300	4.0	*	3.0[c]	240	4.1	*	2.4[c]
SEM	±0.26 (32 df)	±0.35 (24 df)	±0.30 (32 df)		±0.29 (32 df)	±0.30 (16 df)	±0.23 (32 df)

Table 6.9 Sensory attributes of DEF-BP stored at ambient and accelerated storage conditions

Attribute	Appearance		Flavor		Taste		Consistency		Doneness		Overall acceptability	
Storage condition	Amb	Acc	Amb	Acc	Amb	Acc	Amb	Acc	Amb	Acc	Amb	Acc
Storage period (days)	120	70	120	70	120	70	120	70	120	70	120	70
Control	5.0[a]	5.0[a]	4.2[a]	4.2	4.7	4.7[a]	5.0	5.0	4.9	4.8	3.8	4.2
A-MP	4.2[b]	4.5 [c]	2.8[d]	3.6	4.2	3.9[b]	4.8	5.0	4.3	4.8	3.4	3.6
A-AF	4.3[b]	4.9[ab]	3.1[bcd]	3.7	4.5	4.2[ab]	4.9	5.0	4.8	4.8	3.4	3.7
V-MP	4.3[b]	4.9[ab]	3.6[abc]	3.6	4.1	4.0[b]	4.8	5.0	4.7	4.8	3.4	3.6
V-AF	4.3	4.9[ab]	3.7[ab]	3.7	4.5	4.5[ab]	4.9	5.0	4.8	4.8	3.4	3.8
SEM (40 df)	±0.20	±0.10	±0.27	±0.36	±0.34	±0.23	±0.10		±0.27	±0.10	±0.10	±0.25
Storage period (days)	180	150	180	150	180	150	180	150	180	150	180	150
Control	4.7[a]	4.4	3.8	3.9	4.7	4.7	4.8	4.9	5.0	5.0[a]	3.7	3.9
A-MP	3.7[b]	3.7	3.3	3.3	4.2	3.7	4.8	4.6	3.9	4.3[b]	3.5	3.4
A-AF	4.7[a]	4.2	3.3	3.7	4.3	4.6	4.8	4.7	4.5	4.6[ab]	3.6	3.6
V-MP	4.7[a]	4.1	3.5	3.4	4.1	4.3	4.8	4.6	4.5	4.4[b]	3.4	3.6
V-AF	4.7[a]	4.4	3.6	3.9	4.5	4.6	4.8	4.8	4.9	4.8[ab]	3.6	3.7
SEM (40 df)	±0.30	±0.38	±0.36	±0.29	±0.43	±0.32	±0.10	±0.16	±0.31	±0.15	±0.25	±0.28
Storage period (days)	240	210	240	210	240	210	240	210	240	210	240	210
Control	4.5	4.5	3.7[a]	3.5[a]	3.6	3.6	4.5	4.7	4.0	4.8[a]	3.7[a]	3.7[a]
A-MP	4.0	*	2.1[c]	*	2.9	*	4.4	*	3.2	*	2.7[b]	*
A-AF	4.1	3.9	3.0[ab]	2.1[b]	3.2	3.1	4.4	4.4	3.6	3.5[b]	3.1[b]	2.8[b]
V-MP	4.1	*	3.4[ab]	*	2.9	*	4.5	*	3.5	*	3.1[b]	*
V-AF	4.5	4.1	3.4[ab]	3.1[ab]	3.2	3.2	4.5	4.7	3.7	4.7[a]	3.2[ab]	3.0[ab]
SEM	±0.25 (40 df)	±0.31	±0.25 (40 df)	±0.34 (24 df)	±0.53 (40 df)	±0.50 (24 df)	±0.26 (40 df)	±0.20 (24 df)	±0.53 (40 df)	±0.37	±0.20 (40 df)	±0.25

Storage period (days)	360	300	360	300	360	300	360	300	360	300	360	300
Control	3.9	3.4	3.0	3.0	3.0	3.4	4.6	4.6	4.2	4.3	2.8[ab]	2.4
A-MP	3.4	*	1.8	*	2.6	*	4.4	*	3.3	*	2.1[b]	*
A-AF	3.5	3.2	2.1	2.6	2.2	2.9	4.4	4.3	3.4	4.2	2.2[ab]	2.2
V-MP	3.0	*	2.1	*	2.6	*	4.4	*	3.2	*	2.7[ab]	*
V-AF	3.7	3.3	2.5	2.7	2.9	3.0	3.5	4.4	3.5	4.3	2.8[ab]	2.2
SEM	±0.24	±0.34	±0.37	±0.27	±0.37	±0.45	±0.20	±0.22	±0.41	±0.27	±0.20	±0.30
	(40 df)	(24 df)	(40 df)	(24 df)	(40 df)	(24 df)	(40 df)	(24 df)	(40 df)	(24 df)	(40 df)	(24 dwf)

Notations/abbreviations are same as used in Tables 6.5–6.8*A-MP* air-packed-metallized polyester/ploye thylene, *A-AF* air-packed aluminum foil laminate, V-MP vacuum-packed metallized polyester/ploye thylene, V-AF vacuum-packed aluminum foil laminate

Table 6.10 Test of significance between storage condition under different packaging conditions of DEF-BP

	Amb (27°C and 65% RH)						Acc (38°C and 92% RH)				
	Air-packed		Vacuum-packed				Air-packed		Vacuum-packed		
Storage period (days)	MP	AF	MP	AF	Control	Storage period (days)	MP	AF	MP	AF	Control
	Appearance						Appearance				
0	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	0	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}
120	4.7^{a}	4.7^{a}	4.7^{a}	4.9^{a}	5.0^{a}	70	4.5^{a}	4.9^{a}	4.9^{a}	4.9^{a}	5.0^{a}
180	4.2^{ab}	4.3^{ab}	4.5^{ab}	4.8^{a}	4.6^{b}	150	4.2^{a}	4.4^{a}	4.1^{b}	4.4^{ab}	4.7^{c}
240	4.0^{abc}	4.1^{b}	4.2^{b}	4.4^{b}	4.3^{b}	210	*	3.9^{bc}	*	4.0^{abc}	4.5^{bc}
360	3.5^{c}	3.6^{c}	3.7^{c}	3.8^{c}	4.0^{b}	300	*	3.3^{c}	*	3.2^{c}	4.5^{bc}
SEM	±0.24	±0.24	±0.20	±0.20	±0.34	SEM	±0.28	±0.29	±0.24	±0.21	±0.34
	Flavor						Flavor				
0	3.8^{a}	3.8^{a}	3.8^{a}	3.8^{a}	3.8^{a}	0	3.8^{a}	3.8^{a}	3.8^{a}	3.8^{a}	3.8^{a}
120	3.3^{a}	3.7^{a}	3.5^{a}	3.7^{a}	3.6^{a}	70	3.6^{a}	3.7^{a}	3.7^{a}	3.8^{a}	3.8^{a}
180	3.0^{ab}	3.3^{ab}	3.4^{ab}	3.6^{ab}	3.6^{a}	150	3.4^{a}	3.3^{ab}	3.6^{a}	3.6^{a}	3.7^{a}
240	2.8^{abc}	3.1^{abc}	3.4^{ab}	3.4^{ab}	3.6^{a}	210	*	3.1^{abc}	*	3.4^{a}	3.6^{b}
360	1.8^{c}	2.4^{c}	1.8^{c}	2.1^{c}	3.6^{a}	300	*	2.6^{c}	*	2.6^{a}	3.6^{b}
SEM	±0.37	±0.27	±0.26	±0.24	±0.26	SEM	±0.36	±0.34	±0.24	±0.29	±0.28
	Taste						Taste				
0	4.2^{a}	4.2^{a}	4.2^{a}	4.2^{a}	4.2^{a}	0	4.7^{a}	4.7^{a}	4.7^{a}	4.7^{a}	4.7^{a}
120	4.2^{a}	4.0^{a}	4.1^{a}	4.2^{a}	4.2^{a}	70	3.9^{a}	4.5^{a}	4.3^{a}	4.6^{a}	4.7^{a}
180	4.2^{a}	4.1^{ab}	4.0^{a}	4.1^{a}	4.0^{a}	150	3.8^{a}	4.0^{ab}	4.3^{a}	4.5^{a}	4.7^{a}
240	3.0^{b}	3.2^{bc}	3.0^{b}	3.6^{b}	4.0^{a}	210	*	3.2^{b}	*	3.6^{a}	3.1^{b}
360	2.7^{b}	2.2^{c}	2.6^{b}	2.9^{b}	4.0^{a}	300	*	2.9^{b}	*	3.3^{a}	3.0^{b}
SEM	±0.55	±0.37	±0.48	±0.39	±0.39	SEM	±0.38	±0.37	±0.27	±0.36	±0.39
	Consistency						Consistency				
0	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	0	5.0	5.0	5.0^{a}	5.0^{a}	5.0^{a}
120	4.9^{a}	4.9^{a}	4.9^{a}	4.9^{a}	4.9^{a}	70	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}	5.0^{a}
180	4.8^{a}	4.7^{a}	4.8^{a}	4.9^{a}	4.9^{a}	150	4.6^{b}	4.7^{a}	4.6^{ab}	4.8^{ab}	4.9^{ab}

240	4.4[a]	4.5[ab]	4.5[ab]	4.5[ab]	4.9[a]	210	*	4.7[a]	*	4.6[b]	4.6[ab]
360	4.4[a]	4.0[c]	4.2[c]	4.4[b]	4.6[a]	300	*	4.3[a]	*	4.4[b]	4.6[ab]
SEM	±0.20	±0.20	±0.20	±0.20	±0.20	SEM	±0.15	±0.18	±0.14	±0.14	±0.15
	Doneness						Doneness				
0	4.8[a]	4.8[a]	4.8[a]	4.8[a]	4.8[a]	0	4.8[a]	4.8[a]	4.8[a]	4.8[a]	4.8[a]
120	4.7[a]	4.8[a]	4.5[a]	4.8[a]	4.8[a]	70	4.8[a]	4.8[a]	4.8[a]	4.8[a]	4.7[a]
180	3.9[a]	4.5[ab]	4.3[a]	4.6[ab]	4.6[a]	150	4.4[b]	4.8[a]	4.3[b]	4.6[a]	4.6[ab]
240	3.6[a]	3.8[abc]	3.3[a]	4.0[abc]	4.4[ab]	210	*	4.8[a]	*	4.6[a]	4.2[abc]
360	3.4[a]	3.4[c]	3.2[a]	3.4[c]	4.0[ab]	300	*	4.3[a]	*	4.4[a]	4.0[c]
SEM	±0.42	±0.35	±0.50	±0.35	±0.34	SEM	±0.15	±0.17	±0.14	±0.28	±0.34
	Overall acceptability						Overall acceptability				
0	4.2[a]	4.2[a]	4.2[a]	4.2[a]	4.2[a]	0	4.2[a]	4.2[a]	4.2[a]	4.2[a]	4.2[a]
120	3.5[a]	3.6[a]	3.4[a]	3.6[a]	3.8[a]	70	3.8[a]	4.2[a]	3.6[a]	3.7[a]	3.8[a]
180	3.4[ab]	3.4[ab]	3.4[a]	3.4[a]	3.7[ab]	150	3.6[a]	3.0[a]	3.4[a]	3.6[ab]	3.7[ab]
240	2.8[abc]	3.3[ab]	3.1[a]	3.1[abc]	3.1[a]	210	*	2.8[b]	*	3.1[abc]	3.7[ab]
360	2.4[c]	2.2[c]	2.9[a]	2.8[c]	3.3[b]	300	*	2.2[b]	*	2.3[c]	3.3[b]
SEM	±0.25 (32 df)	±0.22 (32 df)	±0.22 (32 df)	±0.22 (32 df)	±0.24 (32 df)	SEM	±0.31 (16 df)	±0.28 (32 df)	±0.28 (16 df)	±0.27 (32 df)	±0.24 (32 df)

three foods did not change drastically irrespective of packaging materials and storage conditions. The stability in consistency indicated no damage in the amylase activity of the malted ingredients of the GEF-LC and no changes at the molecular level in GEF-RT and DEF-BP during storage. In corroboration with consistency, the scores for doneness were also nearly the same as those of control samples evaluated at various stages of storage. The overall acceptability score for the GEF-LC packed in PP was about 3 on 150 days of storage at ambient and 90 days of storage at accelerated storage conditions, and thus indicating that the food remained acceptable up to that period. The foods packed in AF laminates even after 210 and 150 days of storage at ambient and accelerated storage conditions had acceptability score of 2.9 and 3.0, respectively. The slightly low scores of overall acceptability even for the fresh samples could be due to the characteristic taste and aroma of added vitamins, minerals, shadow nutrients, fish oil, and eggs. Moreover, the foods were not artificially flavored and no sugar or artificial sweeteners were added to them. From the sensory attributes, it can be stated that the storage performance of the GEF-LC was almost similar in PP and MP pouches, and superior in AF laminates. The retention of the freshness of the foods packed in AF was better than that of those packed in MP pouches. Similar observations on storage characteristic of some other nutritious foods consisting of malted cereal, legumes, and milk powder have been reported (Malleshi et al., 1989; Gahlawat and Sehgal, 1994; Bhaskaran et al., 2000). The slight browning of the foods observed during storage may be due to the interaction between the malt sugar and the amino acids (Maillard Reaction) of the cereals, legumes, and milk (Lowry et al., 1990; Belitz et al., 2004; Coghe et al., 2006). However, browning was not severe in all the foods. Only after 240 days of storage at ambient and 150 days of storage at accelerated condition, slight browning was noticed.

None of the foods packed in AF formed lumps even after 210 days of storage in both the storage conditions. However, lump formation was observed in GEF-RT and DEF-BP packed in MP and stored at accelerated storage condition after about 150 days of storage. Similarly, caking of the food was observed in the samples packed in MP and stored at accelerated storage conditions after a storage period of 210 days. Mild off flavor was also recorded in samples stored at accelerated storage conditions after 210 days of storage.

Similar to the flavor, the taste of the food was not affected much on storage. The scores for the taste were more than 4 when it was fresh as well as on storage at both the storage conditions. None of the panelists recorded fish oil flavor during the sensory evaluation. The reconstitution characteristics of the foods were also not affected throughout the storage studies. Based on the sensory qualities, it could be stated that the shelf-life of GEF-LC was about 90 days in PP, 120 days in MP, and 150 days in AF laminates in ambient storage condition.

On the other hand, the shelf-life of GEF-RT and DEF-BP was about 200 days in MP and 300 days in AF laminates at ambient storage condition, respectively. Vacuum packing of the spray-dried foods enhanced the shelf-life by about 90 days.

In general, the shelf-life of a food product is determined largely by the nature of ingredients present in the food and the processing methods followed. In particular,

the moisture and the type of lipid contents of the foods, the packaging materials used, and the storage conditions followed also govern the shelf-life of foods. In this study, the moisture content of GEF-LC, GEF-RT, and DEF-BP was 5.6, 2.8, and 2.3%, respectively, which was well within the critical moisture levels of the foods. Because the critical humidity for the enteral foods was less than 60%, the foods packed in PP and MP absorbed moisture even at ambient storage condition. The moisture pickup was rapid in samples stored at accelerated storage condition. The increase in FFA content during storage is related not only to the amount of lipid content, but also to the nature of the lipid and the lipolytic enzymes present in the food. The development of FFA in GEF-LC may be attributed to active lipases present in the cereal malt and their easy accessibility to the added lipids that contained both saturated and unsaturated fatty acids. Further, the increase in moisture content of the samples packed in PP and MP facilitated FFA formation. However, in case of GEF-RT and DEF-BP, there were no active lipases because the food had undergone heat treatment during processing. Hence, the development of FFA in GEF-RT and DEF-BP could be facilitated by the oxidative rancidity. It is noteworthy that the increase in FFA content of all the three foods was marginal albeit the foods did not contain any externally added antioxidant. Probably, the addition of a-tocopherol as a source of vitamin E and the presence of natural antioxidants in the food materials itself restricted the oxidation of the foods to some extent, thus restricting the FFA formation.

The gum acacia, which forms a small component of the ingredients of the enteral foods formulated in the present study, might have played a role by probably forming a thin matrix over the fat globules and thereby preventing its cleavage (PCR, 1993). Although the DEF-BP contains around 20% oil including fish and soy oils, it did not show any reversion during storage. The gum acacia and popped amaranth probably helped encapsulate the flavor of oils during processing. At present no suitable explanation is forthcoming for this beneficial observation and this aspect needs detailed investigation. The whole egg that formed one of the ingredients in DEF-BP did not affect the sensory as well as the storage qualities even though the sugar contents of the egg were not removed. It may be noted that while manufacturing egg powder, its sugars are separated to prevent browning. Further study is required as to why the egg-based DEF-BP did not show significant browning during storage.

From these observations, it can be concluded that the shelf-life of low-cost enteral food (GEF-LC) is about 3 months in PP pouches, and it could be packed in low-priced flexible packaging materials such as HDPP and HDPE pouches for local distribution, or alternatively it can be packed in AF for longer shelf-life and for wider distribution. Both the ready-to-eat formulations packed in AF laminates may be air- or vacuum-packed. However, vacuum packing did not offer substantial advantages. The foods can be packed in bulk for hospital uses. Alternatively, they can be packed in unit pouches of about 70 g each so that the pouch can be cut open and reconstituted in potable liquid just before feeding the patients. The slightly low sensory score for taste and flavor of the foods may be because of the fact that the foods were not artificially flavored and that no sugar was added. This may not seriously

affect the quality of the food when the food is to be fed through the tube. Alternately, natural food flavors such as banana, mango, strawberry, ice cream, vanilla, and chocolate may be added to improve their sensory acceptability when they need to be fed orally. Addition of 5–10-g sugars per 100 g of food should also enhance the taste and flavor of the foods. Further, the foods can be reconstituted in milk or fruit juice to add variety, to improve palatability, and to increase the nutrient density. Furthermore, the foods can also be served to patients in the form of traditional dishes such as pudding, custard, porridge, etc. to avoid the monotony and also to improve their acceptability. The foods can also be served as a supplemental pureed item in long-term care facilities.

Microbiological Safety

The microbiological profile of enteral foods at various stages of their preparation and the freshly prepared foods was evaluated and the same has been discussed in detail in Chap. 4. As discussed earlier in Chap. 4, the foods were enriched with live lactic acid bacteria and were free from harmful microbes. It may be recalled that in case of GEF-LC, out of total plate count of 7.1×10^6 cfu g^{-1}, about 72% of the mesophilic aerobes were lactics. The total plate counts for freshly prepared GEF-RT and DEF-BP were 5.8×10^6 and 6.0×10^6 cfu g^{-1}, respectively, of which 90% of the mesophilic aerobes were live lactics. It may also be recalled that the foods were safe for consumption up to 6 h after preparation.

The changes in microbiological profile at the terminal stage of storage are presented in Table 6.11. During storage, a gradual decrease in total plate count with a marginal decrease in mesophilic spore formers was observed in all the three foods packed in PP, MP, and AF. The GEF-RT packed in MP pouches and stored in accelerated storage condition had slightly higher number of mesophilic spore formers than those stored in ambient storage condition. On the other hand, the vacuum-packed (MP and AF) samples of DEF-BP showed slightly higher number of yeast and mold growth in accelerated storage condition than their counterpart stored in ambient storage condition. Interestingly, the LAB survived the storage period, and the TPC of samples stored at accelerated conditions was lower than that of the samples stored at ambient conditions. The packaging materials exerted very little influence on microflora during storage.

About 60–70% of the LAB survived the spray drying process. Corcoran et al. (2004) studied the survival of spray-dried lactics in the presence of prebiotics and reported that about 50% of lactics survived the spray drying process. Desmond et al. (2002) assessed the protective effect of gum acacia (GA) on the performance of *Lactobacillus paracasei* NFBC 338 species during spray drying, subsequent storage, and exposure of the culture to porcine gastric juice. Their data indicated that GA had applications in the protection of probiotic cultures during drying, storage, and gastric transit. They suggested GA treatment for the manufacture of probiotic-containing powders for efficient probiotic delivery to the host gastrointestinal tract.

Table 6.11 Microbial profile of fresh and stored enteral foods

			Microflora (log_{10} cfu g^{-1})							
			Mesophilic aerobes		Lactics		Yeast and molds		Mesophilic spore formers	
Foods	Packaging condition	Packaging materials	Amb	Acc	Amb	Acc	Amb	Acc	Amb	Acc
GEF-LC	Air	Fresh	7.1		5.2		3.0		3.9	
		Stored								
		PP	6.8	6.1	4.7	3.8	2.3	2.0	3.7	3.7
		MP	5.7	5.3	4.0	3.6	2.4	2.1	3.3	3.0
		AF	4.9	4.6	4.0	3.6	2.1	2.0	3.3	3.0
GEF-RT	Air	Fresh	5.8		5.5		0.8		4.6	
		Stored								
		MP	5.5	5.3	5.1	5.0	0.8	1.0	4.4	4.5
		AF	5.5	5.3	5.0	4.8	1.0	1.0	4.4	4.4
DEF-BP	Air	Fresh	6.0		5.4		2.0		4.3	
		Stored								
		MP	5.7	5.3	4.6	4.2	1.0	1.0	4.3	4.2
		AF	5.6	5.3	4.6	4.2	0.7	1.0	4.2	4.0
	Vacuum	MP	5.1	4.7	4.6	4.1	0.6	0.7	4.0	4.0
		AF	5.0	4.7	4.6	4.1	0.6	0.8	4.0	4.0

In our study the presence of gum acacia might have contributed to the considerable survival of lactic acid bacteria during spray drying and storage.

Lactic acid bacteria offer an array of health benefits in the management of human health and diseases. Some of the established health benefits of LAB are suppression of pathogenic microflora in the intestine, improvement of lactose tolerance, prevention of bacterial translocation, enhancement of immune status, and maintenance of gut barrier functions (Gilliland and Speck, 1977; Gilliland, 1990; Alm, 1991; Rolfe, 2000; Sheih et al., 2001; Benkmark, 2005; Rayes et al., 2005; Kaur et al., 2007). Because of the awareness of various health benefits of LAB and other probiotics, a number of specialty foods have been developed that contain these beneficial microorganisms. Further, studies have shown that LAB improves the palatability (Berg, 1992; Patidar and Prajapati, 1997; Katina et al., 2005), flavor, and taste (Makinen and Bigret, 2004) of foods. This might be the reason for widespread popularity of various fermented dishes that are often rich in LAB and other beneficial microorganisms, amongst various ethnic groups throughout the world. Furthermore, Nfzami et al. (2004) have reported that LAB promotes food safety by removal of dietary toxin. In addition, the role of LAB in development of healthy, tasty, and nutritious cereal-based food is well established (Salovaara, 2004; Katina et al., 2005). Hence, it was appropriate to incorporate live LAB in GEF-LC, GEF-RT, and DEF-BP and to evaluate their stability during processing and storage.

Summary and Conclusion

The shelf-life studies and sensory qualities of the enteral foods developed based on natural food ingredients were carried out simultaneously. The sorption behavior of all the three foods was carried out to help select the appropriate packaging materials. The foods were packed in three different packaging materials, namely: polypropylene (PP), metallized polyester (MP) and aluminum foil laminates. The low-cost food was packed in PP, MP, and AF laminate pouches. Both the spray-dried foods (GEF-RT and DEF-BP) were packed in MP and AF pouches. The DEF-BP was air- and vacuum-packed. The GEF-LC and GEF-RT were air-packed. The foods were stored in ambient [(27 ± 2)°C] and [(65 ± 2)% RH)] and accelerated [(38 ± 2)°C] and [(92 ± 2)% RH] storage conditions. The physicochemical characteristics of the stored foods were monitored by determining the changes in moisture contents, FFA, PV, and pH on each withdrawal. A specially trained taste panelist evaluated the sensory qualities of the stored samples on each withdrawal.

The critical moisture and relative humidity (RH) of the GEF-LC were about 8.0 and 60.0%, respectively, and those of GEF-RT and DEF-BP were 6.0 and 44.0%, respectively.

Based on the observations on physicochemical characteristics, microbiological safety, and sensory attributes, it was inferred that the shelf-life of the GEF-LC was about 90 days in PP, 120 days in MP, and about 150 days in AF at ambient storage condition. This type of packaging materials may be appropriate for the GEF-LC for local distribution. However, the shelf-life of this food can be enhanced if packed in other packaging materials such as metallized polyester and aluminum foil laminates. This may be required for wider distribution of the food, though it will add to the cost of the food. The shelf-life of GEF-RT and DEF-BP packed in MP and AF was about 200 and 300 days, respectively, at ambient storage condition.

Although the DEF-BP contained fish oil and egg, it was readily acceptable without perceptible fish flavor during storage, thereby indicating that the long-chain fatty acids of fish oil and soy oil did not undergo reversion during storage. The foods did not show significant browning during storage. Moreover, the consistency of the prepared foods remained acceptable throughout the storage period. The LAB remained active in all the food samples during the storage period. A slight decrease in the microbial load on storage was observed. However, no pathogenic microflora was detected in the food samples of aforementioned shelf-life periods.

In conclusion, the following statements can be made: The low-cost general-cagory enteral food can be packed in low-priced flexible packaging materials such as polypropylene and polyethylene pouches. The shelf-life of low-cost general-cagory enteral food in such packaging materials is about 3 months at ambient storage condition. The spray dried ready-to-eat enteral foods need to be packed in 100% moisture barrier packaging materials such as aluminum foil laminates, glass bottles, or metallic tins. In such packaging materials, the shelf-life of ready-to-eat enteral foods is about 10 months in ambient storage condition. The shelf-life of the enteral foods can be extended to another 3 months if the foods are packed under vacuum condition. The lactic acid bacteria, which were added to all the three formulated enteral foods, remained active throughout the storage period. In sum, the foods exhibited good shelf-life.

Chapter 7
Clinical Trials on Disease-Specific Enteral Foods

Introduction

This chapter describes the clinical trial conducted to evaluate the acceptability, tolerance, and efficacy of the disease-specific enteral food for burn patients (DEF-BP), developed from natural food sources in the present study.

Disease-specific enteral foods are developed to meet the unique nutritional needs of patients suffering from specific diseases such as celiac diseases, liver diseases, kidney diseases, and burn injury, among others. The rationale for developing disease-specific enteral formulae is that standard enteral formula may not be suitable to provide optimal nutritional support to all kinds of patients.

There are several rationales for developing DEF-BP. Burn patients are hypercatabolic and undergo major physiological changes that affect their nutritional status (Mayes et al., 1996; Wolfe, 1996; Akcay et al., 2001). Extensive burn injury causes accelerated tissue breakdown, increased heat elimination, and erosion of body cell mass. These changes alter the whole body protein turnover model. The top portion of Fig. 7.1 illustrates a simplified first order kinetics model of whole body protein turnover (Q), which may be calculated by using the formula: Q=protein intake (I)+protein synthesis (S)=protein catabolism (C)+nitrogen excretion (E). The increased requirements for new protein during injury require functional redistribution of body cell mass. Net catabolism in integumental, muscular, and intestinal mucosal tissue provides precursors for the synthesis of acute phase protein and visceral tissue. As shown at the bottom of Fig. 7.1, about 15g of acute phase proteins are generated with an additional 5–10g involved with leucocytic activity. These changes are accompanied by a rapid depletion of glycogen stores in muscles and liver, a decrease in fat deposits, and an increase in skeletal protein breakdown to supply amino acids for energy production (Blackburn et al., 1977; Gottschlich and Alexander, 1987; Cynober, 1989; Vincent, 2006). As long as wound healing continues, the patient will remain hypermetabolic and will be in need of increased nutritional support. Moreover, burn patients are immunocompromised and are susceptible to various infections. Because of these biochemical and physiological alterations, nutrition demands for burn patients are immense and often challenging to meet (Prelack et al., 2007). The main objectives of the nutrition intervention in

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DOI: 10.1007/978-0-387-79378-8_7, © Springer Science+Business Media, LLC 2009

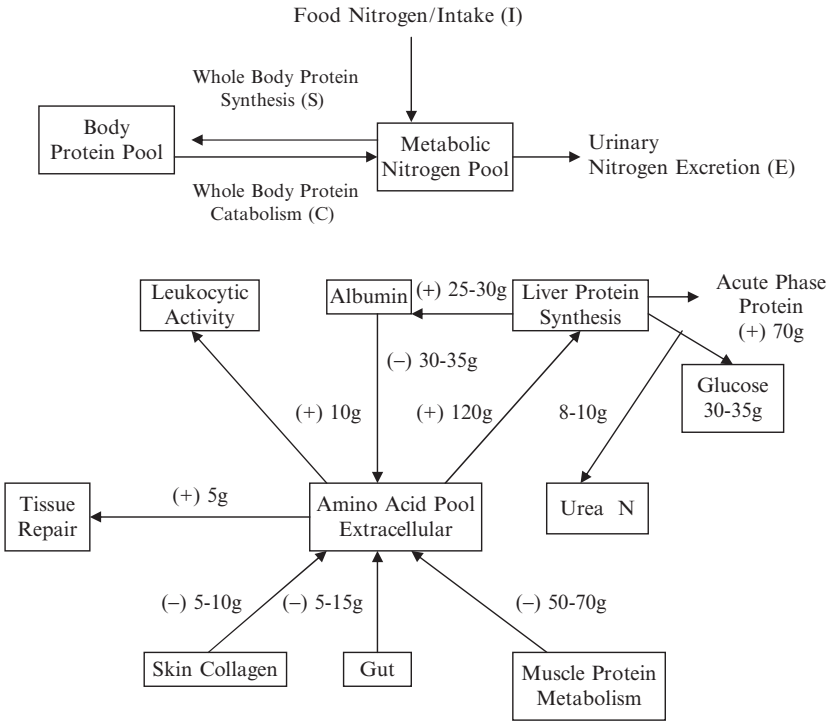


Fig. 7.1 Whole body protein turnover and functional redistribution of body cell mass (source: Blackburn et al., 1977)

the burn patients are to restore fluid and electrolyte balance (as these are lost due to resuscitation), to minimize breakdown of tissue protein to avoid consequences of protein-calorie malnutrition, and to enhance the immunity to prevent sepsis. Therefore, the burn patients require special nutritional support (Curreri et al., 1974; Mochizuki et al., 1984; Gennari et al., 1995; Prelack et al., 2007).

The beneficial effects of energy-dense and high-protein enteral foods for burn patients have been well documented (Gottschlich et al., 1990; Prelack et al., 2007). Accordingly, a number of formulae rich in energy and protein have been developed and evaluated for their clinical efficacy (Smith and Heymsfiekd, 1983; Anderson and Kennedy, 1986; Gottschlich et al., 1990; Montegut and Lowry, 1993). Besides high protein and energy, several studies report the beneficial effect of glutamine, fish oil, and probiotics as anabolic and immune-enhancing nutrients for burn patients (Gottschlich et al., 1990; Chakravarty et al., 2002; Heyland, 2003; Kreymann et al., 2006; Peng et al., 2006).

The DEF-BP developed in the present study is high in energy and protein content, and is enriched with fish oil, glutamine, and lactic acid bacteria. Generally

whenever a new enteral food is developed, its clinical efficacy is carried out in experimental animals, or in human subjects. Several clinical trials relating to the acceptability, tolerance, and clinical efficacy of various disease-specific enteral foods have been carried out throughout the world in animal models or in human subjects (Moore and Jones, 1986; Foley et al., 1990; Kleibeuker and Boersma-Van, 1991; Paulsen and Splett, 1991; Chakravarty et al., 2007). Hence, it was of interest to evaluate the clinical efficacy of DEF-BP in burn patients by controlled clinical trials. This chapter presents the details about clinical trials of DEF-BP conducted on selected burn patients.

Materials and Methods

Ethical Clearance

The ethical clearance from the Ethics Committees of the participating hospitals was obtained for undertaking clinical trials on human subjects. Informed consent from the patient or from their legal guardians was also obtained voluntarily.

The Hospitals

The preliminary clinical trial concerning the acceptability and tolerance of the DEF-BP was carried out on six burn patients in two tertiary care hospitals of India. The result of the preliminary clinical trials was encouraging in terms of acceptability and tolerance of the food. Therefore, controlled clinical trial regarding the acceptability and clinical efficacy of the DEF-BP was conducted on selected burn patients at another tertiary care hospital in India.

Selection of Patients

Forty patients (18 male, 22 female) in the age group of 20–45 years with 30–80% total body surface area burns (TBSA) were selected for the study. As per the hospital records, the patients were admitted within 24h of burn injury. The patients were divided into two groups, and each group consisted of 9 male and 11 female patients. One group received the study diet (DEF-BP) and was assigned as experimental group, while the other group received the diet supplied from hospital kitchen and was designated as controlled group. While grouping the patients, age, TBSA, the extent of third-degree burn, and survival probability were taken into consideration. All the patients were of flame burn injury. Exclusion criteria included

patients with known diabetes, cancer, liver failure, kidney failure, HIV/AIDS, pregnancy, and pediatric patients.

Assessment of the Burn Wound

The burn wounds were assessed in terms of depth and TBSA.

Extent of Burn

The extent of burn depth and the TBSA were assessed according to the method suggested by Luckmann and Sorensen (1987) and Lund and Browder (1944), respectively (Figs. 7.2 and 7.3).

Degree of Burn

The degree of the burn wound of each patient was assessed depending upon the extent of the dermis burn. This was done to decide the need for closure of the wound by grafting. Appendix C shows the varying degree of TBSA burn in two patients.

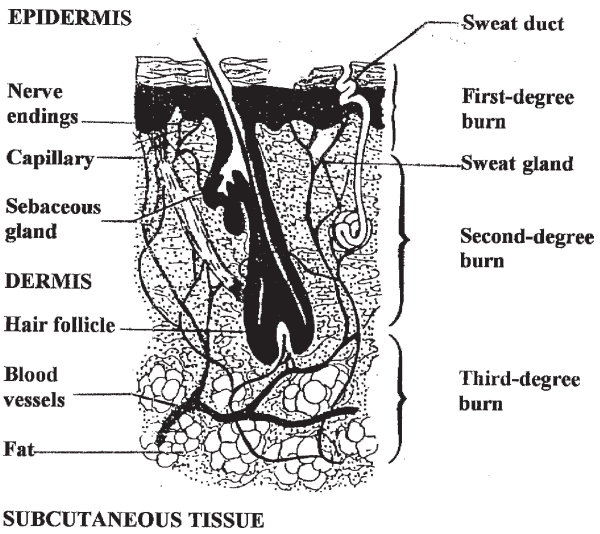
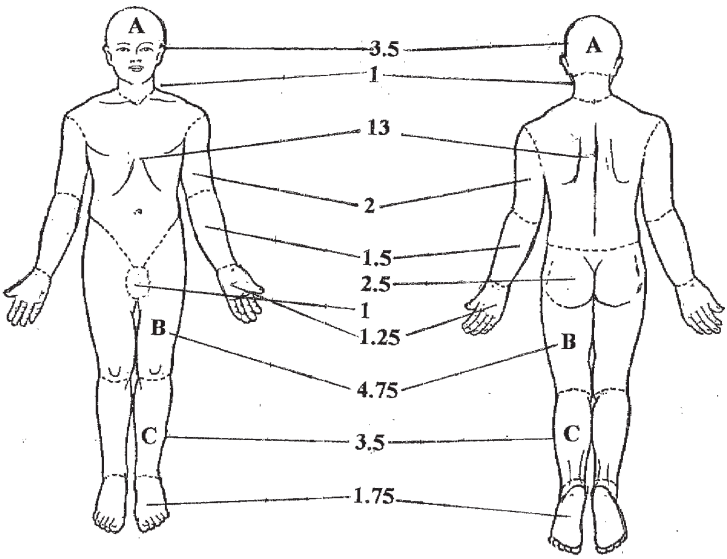


Fig. 7.2 Burn Depth (source: Luckmann and Sorensen, 1987)



A= ½ of Head; B = ½ of One Thigh; C = ½ of One Leg

Age (year)	*Birth*	1	5	10	15	*Adult*
Area						
Head	19	17	13	11	9	7
Neck	2	2	2	2	2	2
A.Trunk*	13	13	13	13	13	13
P.Trunk§	13	13	13	13	13	13
Buttocks	5	5	5	5	5	5
Genitalia	1	1	1	1	1	1
Upper Arms	8	8	8	8	8	8
Forearms	6	6	6	6	6	6
Hands	5	5	5	5	5	5
Thighs	11	13	16	17	18	19
Legs	10	10	11	12	13	14
Feet	7	7	7	7	7	7
Total	100	100	100	100	100	100

A. Trunk (Anterior trunk): Without neck and genitalia*
P. Trunk§ (Posterior trunk): Without neck and buttocks

Fig. 7.3 Estimation of total body surface area burn (source: Lund and Browder, 1944)

Wound Care

Soon after admission, the burn patients were resuscitated from shock. The loose and devitalized tissues were removed. The wounds were treated with primary excision, and 0.5% aqueous silver nitrate solution was applied on the wounds as a topical antibacterial agent.

Survival Probability

The survival probability of each patient was determined based on age of the patients and percent of burn according to the method suggested by Feller et al. (1980).

Nutritional Support

Determination of Energy and Protein Requirement

The energy requirement of each patient was determined according to Curreri's (1979) formula:

Kcal per day = 25× normal body wt (kg) + 40(% TBSA),
(taking a maximum of 50% TBSA).

Protein was provided at the level of 1.5–2.0 g kg^{-1} ideal body weight per day, because most of the studies have shown that this level is safe and adequate to meet the protein requirement (Wolf, 1996).

Feeding Module

The patients of both the groups were able to consume food orally and denied tube feeding. Therefore, the control and experimental groups received nutritional support orally. The experimental group received only DEF-BP as a total nutritional support. The food was dispersed in boiled and cooled water or milk (as per the preference of the patients) to uniform porridge consistency (30–35% solid concentration) and served in 8–10 equal servings of 200–250mL each, starting from 5 A.M. to about 11 P.M. While preparing the diet, about 5–10% sucrose was added to enhance the calorie density and to improve the taste. Reconstituting the food with milk further enhanced the nutrient density. The quantity of the food was increased gradually to attain patients' optimal calorie requirement as per the calculations. The control group received the special hospital diet designed for burn patients from the hospital kitchen. This diet is based on white bread, cooked rice, dhal (decorticated cooked legumes seasoned with oil), vegetables, milk, egg, and meat. This hospital diet matched with the study diet in terms of energy, protein, vitamins, and mineral contents but was devoid of added glutamine, fish oil, and lactic acid bacteria.

Feeding was initiated within 24h of admission, because early feeding was reported to reduce subsequent metabolic rate, and decrease secretion of metabolic hormones and atrophy of the gut mucosal cells (Bell et al., 1986; Saito et al., 1987; Gottschlich et al., 1990).

Outcome Measures

The Protocol

The clinical investigations were conducted and recorded following a specially designed protocol for burn patients. The salient features of this protocol are summarized in Appendix B.

Energy Intake

The total food intake of each patient was recorded everyday to compute the energy and protein intake.

Acceptability and Tolerance

Acceptability and tolerance of the foods by the patients were closely monitored, and occurrence of diarrhea, vomiting, gastrointestinal ileus, or any other discomfort was observed and recorded throughout the study period. Passage of more than three large loose stools (200 mL and above) in a day was considered as diarrhea, whereas stoppage of defecation for 24–30 h was considered as constipation. The general response about the food by the patients was also recorded.

Stress Ulcer Examination

Endoscopic examination was carried out in all the patients for curling ulcer or gastritis by gastroenterologists, because H–2 blockers were not given to the patients.

Anthropometric Indices

Precise measurement of weight for each patient was recorded on admission. Thereafter, weights were recorded at every 5-days interval. Preburn weight of each patient was recorded to compute weight loss. Preburn height was also recorded for all patients to compute BMI.

Hematological Indices

About 1mL of venous blood was collected directly into dry tubes containing recrystallized EDTA (2mg mL^{-1} blood) and used for determination of hematological parameters.

Hemoglobin (Hb)

Twenty microliters of the blood was added to 5-mL cyanmethemoglobin reagent, and the color was read at 540 nm against the blank. The Hb content was expressed as gram percent (Van Kampen and Zijlstra, 1961).

Red Blood Cells (RBC)

Twenty microliters of blood was drawn into 4-mL RBC diluting fluid, mixed well, charged into the Levy counting chamber with improved Neubauer ruling, and counted as per the method of Van Kampen and Zijlstra (1961).

White Blood Cells (WBC)

One in 20 dilution of the blood was made with WBC diluting fluid and mixed well, and the total count was read using a hemocytometer. The differential WBC count was determined using the Leishman's staining method of Van Kampen and Zijlstra (1961).

Packed Cell Volume (PCV)

The PCV or hematocrit was determined by microhematocrit method. Blood taken into microcapillaries was centrifuged at 12,000 rpm for 5 min. The PCV was recorded directly based on the calibration of the capillary tubes. The values were expressed on percentage basis (Bharucha et al., 1976).

Mean Corpuscular Volume (MCV)

The MCV was calculated from the PCV and RBC count as per Bharucha et al. (1976) using the formula:

MCV (cmm) = PCV/red cell count (in millions) × 10.

Mean Corpuscular Hemoglobin (MCH)

The average amount of Hb per cell in all the red cells was calculated according to Bharucha et al. (1976) using the formula:

MCH (Pg) = Hb (grams per 100 mL)/RBC (in millions) ×10.

MCH Concentration (MCHC)

The portion of the average red blood cell containing average concentration of Hb was calculated using the formula of Bharucha et al. (1976).

MCHC (grams per 100 mL) = Hb (g)/PCV × 100.

Biochemical Indices

Blood Analyses

An aliquot of blood (8 mL) was collected in clear dry tubes from each patient. The serum was separated at the earliest and stored at subzero temperature. The different biochemical indices, viz., glucose, serum creatinine, total protein, albumin, globulin, urea, alkaline phosphatase, blood electrolytes (potassium and sodium), and liver function tests were carried out using the diagnostic kits (Stangen Immuno Diagnostics, India) following the standard methods.

Urine Analyses

Twenty-four hour urine output was collected (6.00 A.M. to 6.00 A.M.) from each patient separately and the total volume was recorded. Urinary total protein and creatinine were estimated periodically (5-days interval) using the diagnostic kits (Stangen Immuno Diagnostics, India).

Stool Analyses

Lactose content in the stools was analyzed to examine lactose intolerance.

Assessment of Immune Status

Total Lymphocyte Count

Total lymphocyte count (TLC) was calculated using the following formula:

Total lymphocyte count (TLC) = percent lymphocytes × WBC/100.

Humoral Immunity (HI)

Humoral immunity of individual patient was assessed by determining immunoglobulins (IgG, IgA, and IgM) using immunodiffusion plates (Behringwerke AG, Germany). These immunodiffusion plates contain a prepared agar gel in which H-chain-specific antiserum to the respective immunoglobulin is incorporated. The antiserum is produced by immunization of sheep and goats. IgM and IgA were determined using undiluted serum. For the determination of IgG, the sera were tested and the control serum used was diluted to 1:10 with isotonic saline. Well no. 1 was filled with 5μL of control serum. Wells numbered 2–12 were each filled with 5μL of the respective sera under test. The plates were closed tightly and left to stand

at room temperature. At the end of the given diffusion time (minimum 50 h for IgG and IgA and 80 h for IgM), the diameters of the precipitation rings were measured accurately to 0.1 mm using a suitable calibrated Behring scale, and serum immunoglobulins were determined.

Cell-Mediated Immunity (CMI)

Cell mediated immunity (CMI) is an immune response that does not involve antibodies but rather involves the activation of macrophages, natural killer cells (NK), and antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen. Following burn injury, the CMI is impaired, and proper nutritional support helps boosting the CMI. Hence, CMI was evaluated by lymphocyte migration inhibition test in all the patients toward the end of the study.

Lymphocyte Migration Inhibition Test (LMIT)

The LMIT has been used as one of the measures of cell-mediated immune response. It demonstrates the ability of T-cells (from inoculated individuals) to produce lymphokines upon a second exposure to the specific antigen. The specific lymphokine, which is effective in this assay, is the lymphocyte migration inhibition factor that inhibits the migration of the lymphocytes from the focus of infection.

One millimeter of heparinized blood was layered over 3 mL of lymphocyte preparation and incubated at 37°C till the lymphocytes separated as a layer. The lymphocyte layer was aspirated with a sterile Pasteur pipette. The lymphocytes suspension was washed with TC 199 medium and the final cell pellet was suspended in 0.5 mL of the same medium. This was filled in sterile siliconized, non-heparinized capillary tubes (20-μm diameter), one end of which was sealed using plaster of Paris. The tubes were then centrifuged (500 rpm) for 3 min and cut using a diamond pencil at the packed cell interface.

LMIT plates with wells were taken, and the upper surface of the wall of the wells was greased and a small amount of grease was also deposited inside of the well. The cell-packed capillary tube was then fixed in the grease with the sealed end. The well was then filled with TC199 medium, and a cover slip was placed carefully so as to prevent any air bubbles from entering into the wells. The control wells were filled only with the medium, whereas in the test wells 1μg mL^{-1} of phytohemagglutinin (PHA) was added. The plates were then placed on an even surface and incubated at 37°C in 5% CO_2 and 95% humidified air for 18 h. The area of migration was quantitated by measuring the horizontal and vertical diameters using a microscope fitted with an occlumeter.

The effect of antigen on migration of the lymphocytes (cells) out of the capillary tubes was assessed by calculating the migration in presence of antigen as a percentage of that obtained in its absence (David, 1966) by using the following formula:

Migration (%) = average area of migration with antigen/average area of migration without antigen × 100,

Migration inhibition (%) = 100 – (average area of migration with antigen/ average area of migration without antigen).

Grafting

Split-thickness grafting was carried out in the cases wherever necessary after scar separation by the medical personnel, and percent of graft acceptance or graft rejection was observed and recorded.

Statistical Analyses

The data generated were statistically analyzed by using the T-test for equality of means for two populations and by paired T-test for two populations with unknown variables (Steel and Torrie, 1980).

Results

The information about the physical and physiological status of the burn patients included in the study is presented in Table 7.1. The patients were in the age group of 20–45 years, and 55% of them constituted female patients of child-bearing age. Their body mass index ranged from 17.8 to 30.5 kg m^{-2}. All patients were of flame burn injury but none of them had inhalation injury.

TBSA and Survival Probability

The average TBSA of the control (41.3 ± 7.7) and the experimental (42.5 ± 10.4) groups were comparable. However, the extent of the third-degree burns in the

Table 7.1 Characteristics of the patients

Characteristics	Control *N*=20	Experimental *N*=20
Female (no.)	11	11
Age (years)	30.7 ± 7.5 (20–40)	36.2 ± 7.8 (25–44)
Preburn weight (Kg)	63.3 ± 3 (50–60)	62.2 ± 8.1 (50–75)
Body mass index (Kg m^{-2})	22.3 ± 3.1 (17.8–26.7)	25.9 ± 3.4 (21.6–30.5)
Total body surface area burn (%)	41.3 ± 7.7 (40–81)	42.5 ± 9.4 (39.5–80)
Extent of third-degree burn	12.9 ± 5.1 (5–20)	16 ± 5.1 (9–21)
Survival probability (%)	0.9 ± 0.2 (0.56–0.95)	0.82 ± 0.14 (0.56–0.96)

Figures in parentheses indicate the actual range

experimental group (16% ± 5%) was slightly higher than in the control group (12.9% ± 5%). The calculated survival probability was more than 0.9 for majority of the patients except one patient in the control group, and three patients in the experimental group had lower survival probability ranging from 0.8 to 0.6. The survival probability, which is mainly related to the TBSA and age of the patients, exhibited a negative correlation for the control ($r = -0.96$) and for the experimental ($r = -0.82$) groups at 5% significant level.

Nutrient Intake

The calorie requirements of the patients calculated as per the Curreri's formula (Curreri, 1979) ranged from 2,700 to 3,875 kcal day^{-1}. The average calorie requirement and the actual intake by the patients of both the groups are summarized in Fig. 7.4. Although the food was provided ad libitum, the food intake by most of the patients

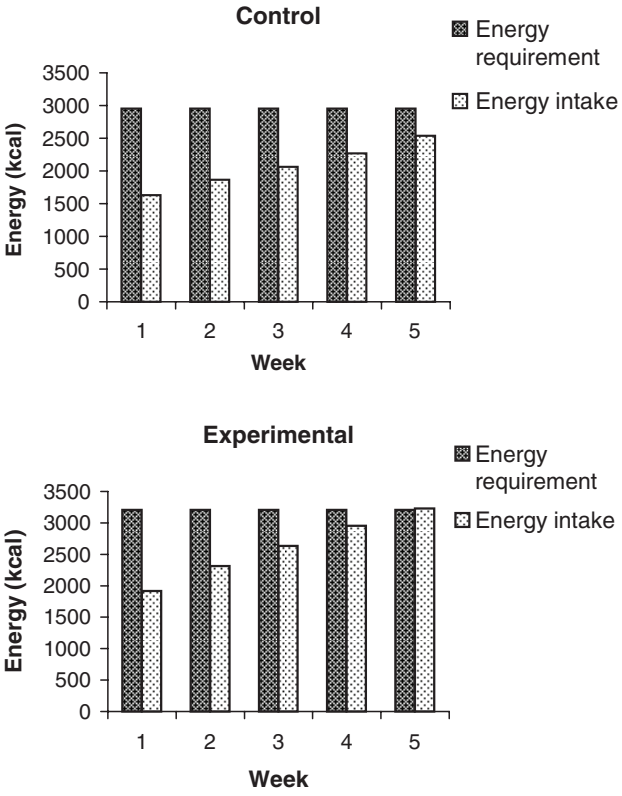


Fig. 7.4 Energy requirement and energy intake of the patients

at the initial stages was suboptimal. After overcoming the initial shock and trauma, the intake was increased gradually to provide the predetermined quantity. During the third week, all the patients in the experimental group could derive 91–100% of their calorie requirement, while patients in the control group could derive 75–80% of their calorie requirement. In fact, the patients in the experimental group started liking the food and consumed slightly higher quantity than the predetermined quantity. But in case of the control group, most of the patients could not ingest food to a desirable extent, because it was provided in the form of rice, bread, dhal, vegetables, milk, etc. The nutritional information of the two dietary regimens and the DEF-BP (reconstituted in skim milk with addition of 5–10-g sugar) has been presented in Tables 7.2 and 7.3, respectively. These observations on the difficulty in providing adequate nutrition to burn patients by means of normal hospital diets have been reported earlier (Gormican et al., 1973; Chiarelli et al., 1990; Talbot, 1990). Because the experimental diet was calorie-dense and contained adequate quantity of protein and other protective nutrients, the patients of this group received about 3,000–4,000 kcal, 100–125-g protein, and adequate levels of vitamins and minerals.

The total food intake by the experimental group was 1,500–2,000 mL, which amounted to 500–600g of food solids. Additionally, patients in the experimental group received 20–24-g L-glutamine, 25–30-g fish oil, and $2{,}700 \times 10^6$ fu^3 of lactic acid bacteria per day. Although patients in the control group could derive 2,300–2,900 kcal and 80–90 g of protein per day, their diet was devoid of L-glutamine, fish oil, and added lactic acid bacteria. The patients readily accepted the DEF-BP and no incidence of nausea, vomiting, and ileus was reported. None of the patients in both the groups developed constipation during the study period. The acceptability and tolerance of the DEF-BP could be attributed to the pleasant taste and aroma of the malted ingredients and the favorable osmotic load. The smooth semisolid consistency

Table 7.2 Nutritional information of control and experimental diet

Diet	Control	Experimental	% Calories
Protein (g%)	23	23	19–20
Fat (g%)	18	18[a]	30–33
Carbohydrate (g%)	58	58	48–50
Energy (kcal per 100g)	486	486	
Vitamins+minerals (g%)	2.4	2.5	
Total dietary fiber (g%)	3.6	5.0	
Soluble dietary fiber (g%)	NA	2.3	
Insoluble dietary fiber	NA	2.7	
Calcium (mg%)	300	300	
Phosphorous (mg%)	310	314	
LAB (cfug^{-1})	NA	5.4×10^6	
BCAA (g per 100 g of protein)	NA	13.31	
Glutamine (g per 100 g of protein)	NA	20.74	
Volume to meet 100 % RDA of vitamins and minerals (30% conc.)	NA	1,200 mL	

NA not available
[a]5-g fish oil

Table 7.3 Nutritional information of reconstituted DEF-BP at 30% solid content

Kilocalories L^{-1}	1,600.0
Protein (% calories)	19–20
Carbohydrate (% calories)	48–50
Fat (% calories)	30–33
Protein (g L^{-1})	69.0
Carbohydrate (g L^{-1})	174.0
Fat (g L^{-1})	54.0
Cal:N	145:0
Non protein calorie:N	120:1
Sodium (mg L^{-1})	330.0
Potassium (mg L^{-1})	630.0
Osmolality (mOsmol kg^{-1} water)	500.0
Renal solute load (mOsmol L^{-1})	219.0
Volume to meet % of RDA for vitamins and minerals (mL)	1,200
Protein source	Egg, milk, soy, legume
Carbohydrate source	Cereal, milk, legume, sugar
Fat source	Fish oil, soy oil, coconut oil
Intended use	Burns, trauma, sepsis, AIDS, patients at hypercatabolic state

of the DEF-BP facilitated the easy oral intake in those patients with burn injury around the lips.

Changes in Body Weight

As expected, all the patients experienced weight loss after the burn injury. There was a gradual loss in weight up to 10 days in the experimental group and after that a slight stabilization of weight was recorded. Gradual decrease in the body weight continued till the third week in case of the control group, after which weight maintenance was observed. The average weight loss (6.9%) in the experimental group was significantly lower ($p<0.05$) than that in the control group (16.3%). A maximum of 25% preburn weight loss was recorded in one of the patients in the control group, whereas, the maximum weight loss in the experimental group was around 9.5% (Fig. 7.5).

Urinary Indices

Protein

The total average urine output of the experimental and the control groups per day per patient was about 1,400 mL. The excretion of protein in the urine was considerably higher in all the patients initially, which was reduced as the feeding progressed.

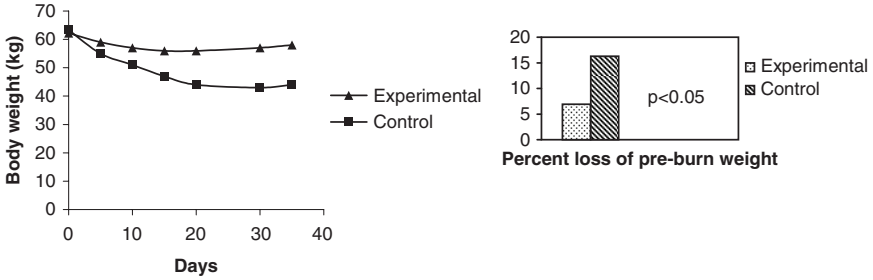


Fig. 7.5 Changes in body weight of the patients

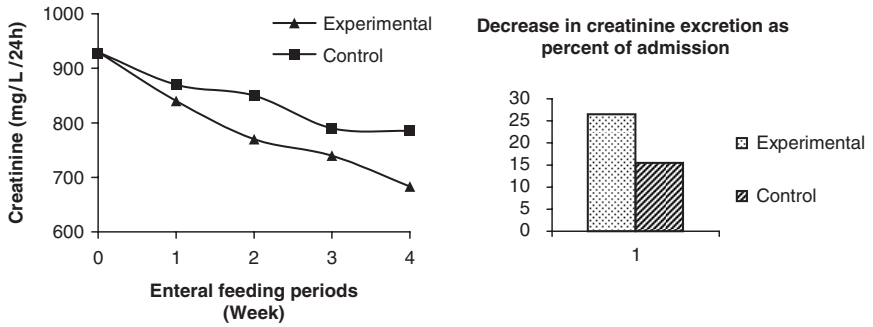


Fig. 7.6 Pattern of urinary creatinine excretion

The marked loss of protein in urine following burn injury may be due to temporary loss of glomerular barrier for protein resulting from severe stress response. The average urinary protein excretion was significantly higher ($p<0.05$) in the control group than in the experimental group. Significant reduction in the urinary protein excretion observed in the experimental group indicated the recovery of the kidney functions faster than in the control group.

Creatinine

The pattern of urinary creatinine excretion indicated that at the initial stage, the 24-h creatinine excretion was almost same in both the groups (930 ± 87mg L^{-1} day^{-1}) with a gradual decrease thereafter. Toward the termination of this study, creatinine excretion was 683 ± 84mg L^{-1} day^{-1} for the experimental group and 785 ± 85g L^{-1} day^{-1} for the control group, thus indicating that the musculature loss was lower in the experimental group than in the control group (Fig. 7.6)

Hematological and Biochemical Indices

The results of the hematological and biochemical indices are as follows.

Hematological

The various hematological and biochemical indices of all the patients receiving experimental and control diets at the initial and terminal stages of the study are presented in Tables 7.4 and 7.5. A slight increase in the hemoglobin percentage (about 14%) was observed in all the patients following immediate burn injury. This could be due to the immediate fluid loss following injury. Hemoglobin level came to the normal range in all the patients toward the termination of the study. The changes in RBC counts did not show much difference between the control ((3.58±0.8) per mm^3) and the experimental groups ((3.71 ± 0.6) per mm^3) at the end of the study, which was marginally significant ($p < 0.1$). As expected, the count of

Table 7.4 Hematological and biochemical indices of blood/serum of patients

	Control (C) group		
Parameters	I	T	I vs. T (*p*)
Glucose (mg%)	80 ± 8.4	84.3 ± 12.1	NS
Urea nitrogen (mg%)	13.0 ± 8.2	14.8 ± 7.3	0.05
Creatinine (mg%)	0.8 ± 0.1	0.9 ± 0.1	NS
T. protein (g%)	7.4 ± 0.7	6.9 ± 1.3	NS
Albumin (g%)	4.1 ± 0.3	3.1 ± 0.6	0.05
Globulin (g%)	3.4 ± 0.8	3.7 ± 0.9	NS
A/G	1.3 ± 0.5	0.8 ± 0.2	0.05
Na (mEq L^{-1})	133.5 ± 5.4	131.8 ± 9.1	NS
K (mEq L^{-1})	4.7 ± 0.7	3.9 ± 0.7	0.01
Bilirubin (m g%)	0.8 ± 0.1	0.8 ± 1.0	0.05
Alkaline phosphatase (unit%)	15.5 ± 7.7	10.3 ± 3.2	0.05
SGPT (unit mL^{-1})	281.0 ± 129.9	134.5 ± 40.9	0.05
Hemoglobin (g%)	13.2 ± 1.7	11.83 ± 2.64	NS
RBC (mg mm^{-3})	4.1 ± 0.7	3.6 ± 0.8	NS
WBC (mg mm^{-3})	11,716 ± 313	9,316 ± 140	0.05
Hematocrit (vol%)	36.8 ± 6.5	34.7 ± 6.7	0.05
MCV (cmm)	8.3 ± 2.3	9.7 ± 0.7	0.1
MCH (pg)	33 ± 4	33 ± 5	NS
MCHC (%)	36.2 ± 5.2	34.0 ± 31	0.1
Neutrophils (%)	73.3 ± 15.6	70.3 ± 6.0	0.1
Lymphocytes (%)	20.8 ± 138	25.3 ± 6.9	0.05
Monocytes (%)	2.5 ± 1.4	1.5 ± 1	NS
Eosinophils (%)	3.3 ± 2.3	2.8 ± 2.1	NS

Vandanberg reaction was negative for both the groups Basophil count was nil for both the groups

I at the initiation of study, *T* at the termination of the study, *NS* not Significant, *P* probability

Table 7.5 Hematological and biochemical indices of blood/serum of patients

	Experimental (E) group			
Parameters	I	T	I vs. T (*p*)	C vs. E (*p*)
Glucose (mg%)	82.5 ± 15.1	93.3 ± 6.3	0.0	0.05
Urea nitrogen (mg%)	14.3 ± 7.9	12.9 ± 9.3	NS	NS
Creatinine (mg%)	2.7 ± 4.56	0.8 ± 0.4	0.05	NS
T. protein (g%)	7.1 ± 1.1	7.5 ± 0.6	NS	0.05
Albumin (g%)	3.3 ± 0.4	4.7 ± 0.5	0.05	0.05
Globulin (g%)	3.8 ± 0.4	2.8 ± 0.4	0.05	0.05
A/G	1.0 ± 0.3	1.6 ± 0.2	0.05	0.05
Na ($mEqL^{-1}$)	130.8 ± 9.5	137 ± 7.4	0.05	NS
K ($mEqL^{-1}$)	4.1 ± 1.3	41 ± 0.7	NS	NS
Bilirubin (mg%)	0.8 ± 0.1	0.8 ± 0.1	NS	NS
Alkaline phosphatases (unit %)	21.8 ± 13.5	6.8 ± 01	0.05	0.05
SGPT ($unitmL^{-1}$)	198.3 ± 135.7	45 ± 11.1	0.05	0.05
Hemoglobin (g%)	14.0 ± 2.9	11.7 ± 2.0	NS	NS
RBC ($mgmm^{-3}$)	3.6 ± 0.5	3.7 ± 0.6	NS	NS
WBC ($mgmm^{-3}$)	10,950 ± 2,090	8,316 ± 12,350	0.05	NS
Hematocrit (vol%)	34.0 ± 2.8	36.3 ± 4.6	0.05	NS
MCV (cmm)	9.6 ± 0.8	9.8 ± 0.5	NS	0.1
MCH (pg)	40 ± 0.9	31 ± 1	0.05	NS
MCHC (%)	41.4 ± 9.7	31.9 ± 1.6	0.05	NS
Neutrophils (%)	69.1 ± 3.1	63.8 ± 4.3	0.05	0.05
Lymphocytes (%)	26.6 ± 2.7	31.6 ± 2.6	0.05	0.05
Monocytes (%)	2.0 ± 0.6	1.7 ± 0.5	NS	NS
Eosinophils (%)	2.2 ± 0.8	3.0 ± 2.0	NS	NS

Vandanberg reaction was negative for both the groups Basophil count was nil for both the groups
I at the initiation of study, *T* at the termination of the study, *NS* not Significant, *P* probability

the WBC was higher by about 25% in most of the patients in the initial stage, which gradually decreased reaching to a normal range toward the end of the study.

There were no significant differences between the initial and final levels of MCV ((8.3 ± 0.81 vs. 9.7 ± 0.7)mm^3) and ((9.6 ± 0.8 vs. 9.8 ± 0.57)mm^3) and MCH ((33 ± 4 vs. 33 ± 5)pg and (40 ± 9 vs. 31 ± 1)pg) for the control and the experimental groups, respectively. The better blood picture of the experimental group than the control group, observed in terms of WBC and RBC, reflects the beneficial effect of superior nutritional support through the DEF-BP diet. The percent of neutrophils decreased from an average of 73.3% ± 15.6% to 70.3% ± 6.0% for the control group and 69.1% ± 3.1% to 63.8% ± 4.3% for the experimental group during the course of study. Similarly, at the termination of the study, there was a significant ($p < 0.05$) increase in the lymphocyte count of the patients receiving the DEF-BP (31.6% ± 2.6%) compared with the patients on hospital diet (25.3% ± 6.9%). This showed better immune status of the former than the later group. However, the blood picture with respect to monocytes, basophils, and eosinophils was normal in the control and the experimental groups without exhibiting any immature cells and hemoparasites. Adequate platelet count was also observed in all the patients.

Biochemical

The biochemical indices of the serum assay with special reference to glucose, electrolyte, bilirubin, and SGPT are also presented in Tables 7.4 and 7.5. None of the patients (except one patient in control group) showed hyperglycemia. It was unusual because most of the studies report that hyperglycemia is a common symptom following burn injury. During the course of the study, the blood glucose levels of the control and the experimental group remained in the normal range, although the terminal values were slightly higher than the initial values. At the initiation of this study, the blood urea nitrogen (BUN) for the control group was (13 ± 8.2)mg%, which was slightly lower than at the termination of the study (14.8 ± 7.3)mg%. On the contrary, the initial value for BUN (14.3 ± 7.9)mg% in patients of the experimental group decreased to (12.9 ± 9.3)mg% toward the termination of the study. The blood urea, total protein, and the bilirubin contents were in the normal range throughout the course of the study. In the control group, although the serum albumin level (3.1% ± 0.6%) was in the normal range toward the termination of the study, a rise in globulin level (3.7% ± 0.9%) altered the A/G ratio (0.87 ± 0.15). In case of the experimental group, a rise in serum albumin level (4.7 ± 0.5)g% toward the end of the study brought the A/G ratio to the normal level (1.6 ± 0.2), which was statistically significant when compared with the control group ($p < 0.05$). The serum electrolyte levels in both the groups were in the normal range.

An alteration in liver functions was noticed in all the patients following burn injury. There was an abrupt rise in alkaline phosphatase in the control ((15.5 ± 7.7) unit%) as well as experimental ((21.8 ± 13.5) unit%) groups. The initial average SGPT values for control and experimental groups were (281.0 ± 129.9) and (198.3 ± 137.7) unit%, respectively, which were also considerably higher than the normal range. But at the time of termination of the study, values for alkaline phosphatase ((6.8 ± 0.1)unit%) and SGPT ((45 ± 11.1) unit mL^{-1}) in the experimental group decreased considerably, indicating an early recuperation of liver functions in these patients. Although there was a significant decrease in alkaline phosphatase in the patients belonging to the control group toward the end of the study ((10.3 ± 3.2) unit%), it was still many folds higher than the normal range of 1–3.5 unit%. Similarly, toward the end of the study, though there was 52% decrease in the initial SGPT values of the control group, it was still higher than the normal range. The profile of liver enzymes clearly demonstrated the positive role of the study diet over the hospital diet as far as the liver function was concerned. The test for Venderburg reaction was negative for all the patients in both groups.

Immune Status

Total Lymphocyte Count

The TLC for the experimental group (initial: 2,873 ± 228; terminal: 2,627 ± 405) was significantly higher than that of the control group (initial: 2,190 ± 110; terminal: 2,280 ± 378) albeit adequate for both the groups.

Humoral Immunity

The levels of IgG, IgA, and IgM were assessed at the beginning (within 48 h) and toward the end of the study. Initially marked decreased in IgG, IgM, and IgA was noticed among all the patients included in the study. Substantially higher values of IgG ((1,642 ± 219) $mgdL^{-1}$) and IgA ((287 ± 78) $mgdL^{-1}$), and moderate values of IgM ((142 ± 77) $mgdL^{-1}$) were observed in the experimental group toward the end of the study. However, the IgG (498 ± 348) and IgA ((106 ± 43) $mgdL^{-1}$) level remained below the normal range in the patients of the control group at the time of termination of the study. Further, much higher level of IgM ((181 ± 68) $mgdL^{-1}$) was observed in the control group at the termination of the study than the initial value ($p < 0.05$), though it was within the normal range (Fig. 7.7).

Cell-Mediated Immunity (CMI)

The lymphocyte migration inhibition assay has been used by several workers as a measure of CMI (Pathki, 1987). The CMI is mediated by the activity of the thymus-derived lymphocytes and is expressed by elaboration of lymphokines that influence effector cell population or by T-cell-mediated lysis of virus-infected cells. The data on the cell-mediated immunity measured in terms of lymphocyte migration inhibition capacity of the inoculated blood with specific mitogen PHA (phytohemagglutinin) and carried out at the terminal stage of the feeding trials revealed a better cell-mediated immunity in terms of higher migration (81.56% ± 16.4%) of lymphocytes in the experimental group than in the control group (11.05% ± 15.6%). Table 7.6 and Fig. 7.8 show the cell-mediated immune responses in both the groups.

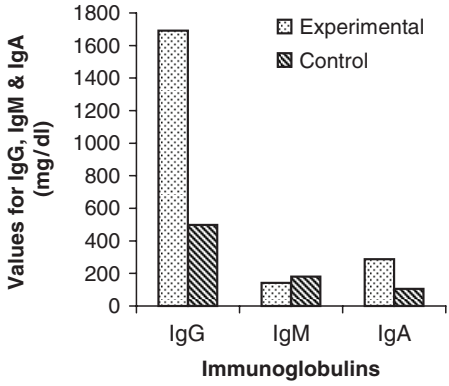


Fig. 7.7 Immunoglobulin levels of the patients at the termination of the study

Table 7.6 Percent migration and migration inhibition (in vitro) in control and experimental groups

	Control	Experimental
Area under migration without antigen (mm)	6.18 ± 1.9	6.91 ± 1.9
Area under migration with antigen	0.49 ± 0.7	5.59 ± 1.5
Migration (%)	11.05 ± 15.6	81.56 ± 16.4
Migration inhibition (%)	88.95 ± 15.5	18.44 ± 16.4

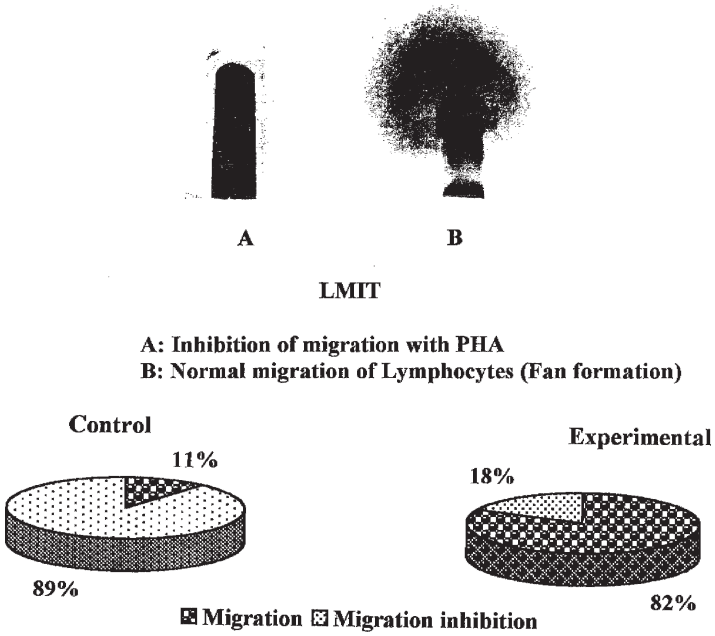


Fig. 7.8 Cell-mediated immunity of the patients

Stress Ulcer

Endoscopic examination revealed restoration of normal gastrointestinal mucosa without showing the incidence of stress or curling ulcer in the experimental group, whereas the incidence of curling ulcer was prominently visible in two patients of the control group. Figure 7.9 shows the mucosa of a patient with stress ulcer (A) receiving hospital diet and normal mucosa of another patient (B) receiving DEF-BP, respectively.

Microscopic examination of the stool and urine were negative for the presence of pus cell, occult blood, and also for lactose in stool. There was no incidence of gastrointestinal disorder, vomiting, or ileus in any of the patients during the study period.

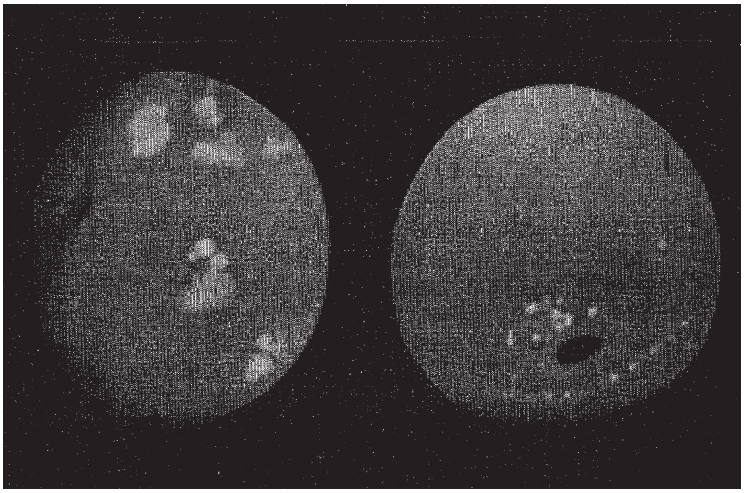


Fig. 7.9 Photomicrographs of gastrointestinal mucosa of two patients: **a** – GI mucosa with stress ulcer, **b** – normal GI mucosa

The patients receiving DEF-BP as total enteral nutritional support recovered faster and were ready for grafting earlier (26–28 days) than the patients receiving hospital diet (35–40days). It was also observed that the success of the graft take was over 90–95% in the experimental group as compared with about 80% of the control group. Further, the experimental group had fewer infectious episodes than the control group.

Discussion

Early enteral nutritional support to burn patients is a desirable therapeutic approach to prevent burn-induced increase in catecholamine and glucagon secretion, thereby leading to an improved control of hypermetabolic state. Following injury, burn-induced hypermetabolism (glycogen depletion) occurs before any adaptive increase in the rate of glucogenesis takes place. Similarly, the proteolysis takes place rapidly causing early muscle wasting and loss of visceral proteins. In addition, during the early period after inflicting burns, insulin concentration decreases probably because of the anti-insulin action of catecholamines. Early supplementation of carbohydrates and other nutrients prevents the inhibition of insulin secretion. This helps better utilization of glucose. It has been observed that an early enteral nutrition support (within 12–24 h of burn injury) is safe and effective (Chiarelli et al., 1990). McDonald et al. (1991) started gastric feeding within 6 h of onset of burn injury on 106 patients with (40 ± 21)%TBSA, and observed that 82% of the patients absorbed at least part of the tube-fed diet on the day of injury, and absorption was increased

up to 95% by the fourth day of hospitalization. However, 16 out of 106 patients frequently vomited. Other investigators have also reported the effectiveness of early enteral nutritional support to burn patients in improving the postburn nutritional status and immunological responses (Marvaki et al., 2001; Hart et al., 2003). It has also been reported that delayed feeding may result in increased increments of gram negative and enteric organisms, sepsis, loss of gut mucosal integrity, increased secretion of catabolic hormones, and severe immunosuppression (Saito et al., 1987; Chiarelli et al., 1990; Germenis et al., 1990; Gottschlich et al., 1990; Gianotti et al., 1995; Marik and Zaloga, 2001). Therefore, it was appropriate that the enteral nutrition support was initiated within 24h of hospitalization in the present study.

It is well understood that a considerable loss of body weight following burn injury is mainly due to muscle protein breakdown (skeletal muscle) and nitrogen loss. The nitrogen loss during first week of injury mainly results from loss of slough or excision, loss of blood and exudates, and the loss of nitrogen due to direct tissue injury and the generalized catabolism (Cuthbertson, 1945). Bell et al. (1986) examined the weight change of 42 adults and pediatric burn patients and reported that 35 patients lost about 10% of preburn weight within a few days of burn injury. They further observed that a dietary regimen consisting of high protein and high calorie prevented weight loss significantly. Later, Lee et al. (2005) reported a significant weight loss following burn injury.

All the patients in this study also lost body weight following burn injury. However, the patients of the experimental group maintained weight from the third week onward, whereas gradual weight loss till fourth week was observed in the patients of the control group. The patients in the control group could not consume enough food because it was served from the hospital kitchen in the form of normal diet (cooked rice, bread, decorticated cooked legumes, meat, vegetables, and some other common foods) with slight modification in consistency, though foods were served ad libitum. On the other hand, because malt-based foods are energy-dense and possess enhanced bioavailability of nutrients compared with normal food, the patients in the experimental group could derive better nutrition in quantity as well as in quality compared with the control group. Additionally, the study diet (DEF-BP) was also enriched with glutamine, fish oil, and probiotics. This might be the reason why the experimental group experienced lesser weight loss than the control group.

Usually, diarrhea and vomiting are common complications in enterally fed patients. The absence of diarrhea and vomiting in both the groups (though feeding was initiated within 24 h of injury) may be due to oral intake of food, which is more physiological than tube feeding. The small quantity of food served in frequent intervals might also have helped tolerate the foods. In addition, the LAB present in the DEF-BP diet and yogurt provided through hospital diet might have also helped control lactose intolerance in both the groups. During the entire course of the feeding period, none of the patients experienced constipation. The presence of dietary fiber in both the diets probably facilitated easy bowel movement in the patients. The beneficial effect of dietary fiber in preventing constipation is well known (Silk, 1993; Eduard, 2004).

Twenty-four hour urinary excretion of creatinine is considered as one of the important indices of nutritional assessment (Heymsfield et al., 1983; Gottschlish et al., 1990; Love and Nguyen, 1999; Yew and Pal, 2006). Creatinine excreted in urine is accepted as an indicator of catabolism of muscle mass (Akcay et al., 2001). Several studies have reported a significant reduction in lean body mass following burn injury (Davis and Fell, 1974; Lee et al., 2005; Vincent, 2006). Observation on 24-h urinary creatinine excretion during the course of this clinical trial revealed that though both the groups lost musculature during hospital stay, the erosion of lean body mass of the experimental group ceased during postburn week 2. On the other hand, the controlled group continued to lose lean body mass till postburn week 3. The better preservation of muscle mass in the experimental group might be due to the supply of balanced amino acids, supplemental glutamine, and adequate energy through the malt-based enteral nutrition received from DEF-BP, which probably facilitated the protein-sparing action.

Because most of the enteral foods are based on hydrolyzed or partially hydrolyzed protein, Trocki et al. (1986) studied the differential effect of intact vs. hydrolyzed protein. They fed 18 guinea pigs with 30% TBSA, a diet containing hydrolyzed whey protein, and 27 similar animals fed with intact whey protein. Both the groups were fed with an isocaloric diet (175kcal $kg^{-1}day^{-1}$) by continuous gastric infusion. After 14 days, the animals were sacrificed. Feeding intact whey protein showed statistically significant benefits in terms of total body weight, cumulative nitrogen balance, liver, gut, and gastrocnemius weight, serum albumin and transferrin level, as compared with the animals fed with hydrolyzed whey protein. Hermansen et al. (2001) also reported other health benefits of intact protein. All the patients included in the current study received intact protein, which they tolerated very well.

Protein catabolism in burn injury increases as energy needs are met by deamination of branched chain amino acids (BCAA) and other amino acids in the generation of carbon skeleton for glucose synthesis in addition to supporting the synthesis of alanine and glutamine. Alanine and glutamine may then undergo transamination forming nonessential amino acids and priority proteins associated with host defense and survival mechanism (Cynober, 1989; Wilmore, 2001). Glutamine serves as a preferred oxidative substrate for enterocytes and may have a vital role in the maintenance of intestinal integrity and function. Fish et al. (1997) showed that glutamine supplemented formulae can be fed either enterally or parenterally.

Many investigators have reported that glutamine supplementation in the feeding regimen of burn patients could abate the degree of glutamine depletion, promote protein synthesis, inhibit protein depletion, reduce infections, improve wound healing, and reduce hospital stay (Peng et al., 2005, 2006; Kreymann et al., 2006; Windle, 2006; Calder, 2007).

In this study, we have observed weight maintenance and better preservation of muscle mass in the experimental group compared with the controlled group. Because the study diet (DEF-BP) was fortified with glutamine, it might have spared a part of deamination of BCAA and other amino acids in the generation of carbon skeleton for glucose synthesis, and thereby helped preservation of muscle mass.

Further, because the DEF-BP is malt-based, the energy density and bioavailabilty of nutrients in DEF-BP are better than that of the nutrients present in the normal diet . Enhanced energy density and improved bioavailibility of nutrients from malt-based food are well reported (Malleshi and Desikachar, 1982; Wisal et al., 2007).

Hyperglycemia is another common phenomena associated with severe burn injury (Riesenman et al., 2007). In this study, none of the patients in the experimental group showed impaired glucose tolerance or hyperglycemia. Only one patient in the controlled group showed hyperglycemia in the mid course of the study, which was well controlled without medication toward the end of the study. The improved overall glucose tolerance observed in most of the patients might be due to the fact that all the patients received adequate amount of dietary fiber through their diets.

More recently, Lazaridou and Biliaderis (2007) have reported the health benefits of b-glucans in reducing blood serum cholesterol and regulating blood glucose levels. Wood (2007) reported that the ability of oat and barley products to attenuate postprandial glycemic and insulinemic response is related to the content of (1→3)-, (1→4)-β-D-glucan (β-glucan) and the viscosity of oat and barley. Therefore, the presence of barley β-glucan in the DEF-BP might have also contributed to regulating the blood glucose level in the experimental group.

The role of dietary lipid in the nutritional support of burn patients has been discussed widely. Mochizuki et al. (1984), based on their detailed investigations on the optimal lipid content of enteral diets in thermally injured pigs, suggested that 5–15% of nonprotein calories from fats was adequate for nutritional support after burn injury.

Alexander et al. (1986) studied the effect of different types of dietary lipids, namely safflower oil (74% linoleic acid), linolenic acid alone, and fish oil (18% EPA) on the overall improvement in the recovery from burn injury in guinea pigs. They reported that the animals fed with diet containing fish oil (18% EPA) showed lesser weight loss, better skeletal muscle mass, lower resting metabolic expenditure, better cell-mediated immune response, better opsonic indices, higher splenic weight, lower adrenal weight, higher serum transferrin, and lower C3 levels than those animals receiving diet containing safflower oil or linoleic acid. Gottshlich and Alexander (1987) suggested that 10–15% of the fat calories should be derived from fish oil or fat rich in n-3 fatty acid.

Garrel et al. (1995) observed that low-fat nutritional solutions were advisable to severe burn patients, because they showed improvement in respiratory function, nutritional status, and metabolic profile. However, they did not find any beneficial effect of fish oil in enteral food for burn patients.

Thierry et al. (2000) observed the effects of the amount and type of fat in the nutritional support on serum insulin-like growth factor (IGF)-I in 23 severely burned patients (>25% total body surface area burned). They also tested the hypothesis that the serum proteolytic activity for insulin-like growth factor binding protein (IGFBP)-3 is a major mechanism for the decreased serum IGF-I observed in these patients. These patients were randomly assigned to three types of nutritional support differing in the amount of energy derived from fat and the presence or absence of fish oil: group I (control), 35% fat; group II, 15% fat; group III, 15% fat with

50% as fish oil. Nutritional support was both parenteral and enteral and was started within 24 h of admission. They reported that in burn injury, serum IGF-I concentrations are sensitive to the amount and type of fat in their nutritional support. The presence of fish oil allowed for a more rapid recovery of serum IGF-I levels. They also concluded that proteolysis of IGFBP-3 may be an important cause of the decreased serum IGF-I values.

Several other investigators reported that nutritional formulae containing immunonutrients such as glutamine, arginine, fish oil, probiotics, RNA, and menhaden oil could exert a significant positive effect on host defense mechanisms after severe injury (Cerra et al., 1983; Alexander et al., 1986; Gottshlich et al., 1990; Gennari et al., 1995; Chakravarty et al., 2002; Bistrian, 2003; Choudhry et al., 2003; Chuntrasakul et al., 2003; McCowen and Bistrian, 2003; Kreymann et al., 2006; Peng et al., 2006; Calder, 2007). Better immunological status observed in the experimental group than in the controlled group in the present study may be due to the presence of glutamine, fish oil, and probiotics in the experimental diet (DEF-BP).

Changes in liver functions following burn injury are common (Jeschke et al., 2007). It is well known that the traumatic injury to the liver following burn injury is precipitated by increase in the liver enzymes, especially alkaline phosphatases, SGPT, and SGOT, and good nutritional support enhances the normalization of liver functions (Saleh et al., 2002). It is also known that cirrhotic patients show decreased levels of plasma branched-chain amino acids (BCAA), and supplementation of BCAA helps recover from encephalopathy (Charlton, 2006).

Branched chain amino acids in the diets of septic patients act as caloric substrates, stimulate protein synthesis, reduce protein catabolism, and improve encephalopathy (Jimenez et al., 1991; Bandt and Cynober, 2006; Cynober and Harris, 2006). Branched chain amino acids and ornithine-”-ketoglutarate (OKG) supplementation improve nutritional status and reduce protein hypercatabolism and accelerate wound healing. Metabolites of OKG regulate protein turnover. It was shown that bolus feeding was beneficial in comparison with the continuous infusion with regard to absorption and metabolism of OKG (Bricon et al., 1997).

In the recent years, the relationship between BCAA and immunity has also been discussed. Intravenous administration of BCAA to postsurgical and septic patients showed improved immunity. BCAA may be essential for lymphocyte responsiveness and to support other immune cell functions. However, more research is needed to understand the role of BCAA in improving the immune system (Calder, 2006).

Kakazu et al. (2007) investigated the roles of BCAA in the function of human monocyte-derived dendritic cells (MoDC) in healthy volunteers and hepatitis C virus (HCV) infected cirrhotic patients. Their results showed that extracellular BCAA, especially valine, regulated the maturation and function of MoDC in all subjects. They also observed that an elevated extracellular concentration of valine improved the dendritic cell functions in cirrhotic patients. Their findings provided a rationale for introducing medical nutrition therapy supplemented with BCAA to patients with cirrhosis of liver.

In the present study, we have observed an abrupt rise in liver enzymes in all the patients following burn injury. An early recuperation to liver functions in the experimental group was observed, which was statistically significant while compared with that of the control group. Improvement in liver function in the experimental group may be due to the presence of BCAA and glutamine in the DEF-BP diet. As discussed earlier, the favorable concentration of glutamine in DEF-BP might have spared the deamination of BCAA. Many investigators have reported the role of BCAA in maintaining liver function in both human and animal models (Cerra et al., 1983; Ferency, 1996; Saleh et al., 2002; Charlton, 2006; Jeschke et al., 2007).

It has also been shown that diet containing lactic acid bacteria improves the liver function in patients with encephalopathy by decreasing the pH of the intestine, and thereby facilitating the disposal of ammonia (Ferency, 1996). An improved liver function observed in the patients of the experimental group might be due to the combined effect of glutamine, BCAA, and LAB present in the study diet.

Lactose intolerance is considered as another problem in enterally fed patients. Hence, most of the enteral formulae currently available are lactose-free. One interesting observation during this study was that none of the patients in both the groups experienced lactose intolerance though they consumed milk. The beneficial effects of probiotic and LAB in improving lactose tolerance have been reported (Patidar and Prajapati, 1997). Fermentation of milk by lactobacilli is known to produce compounds such as lactic acid, acetic acid, hydrogen peroxide, and bacteriocins, which are effective against several intestinal pathogens (Soomro et al., 2002).

Therefore, consumption of the food containing lactic acid bacteria such as yogurt and other fermented products is useful in controlling a variety of intestinal disorders including lactose intolerance. Lactic acid bacteria also facilitates the recolonization of intestinal microflora after heavy antibiotic treatment. β-galactosidase of yogurt hydrolyses lactose also. The absence of lactose intolerance observed in both the groups in the present study could be due to abundance of lactic acid bacteria in the DEF-BP diet and yogurt in the control diet.

A number of studies have shown that lactobacilli not only constitute an integral part of the host's gastrointestinal microecology (Yuguchi et al., 1992), but also play an important role in the host's immunoprotection system by increasing specific and nonspecific immune mechanisms (Paubert-Braquet, 1992; Bengmark, 2005). Colonization of the gut by lactobacilli has increased the host's resistance to infection (De Simone et al., 1986; Bengmark and Martindale, 2005). This may be the reason why the experimental group has fewer infections and experienced early wound healing, particulalry, the deep-burn wound healing as compared with the control group.

Improved hematological indices observed in the experimental group with special reference to the hematocrit, TLC, and improved albumin picture may be attributed to the quality of protein, mostly derived from egg and milk. The improved carbohydrate digestibility of malted cereal could have also contributed to this beneficial effect.

The enhanced immune status recorded both in terms of serum immunoglobulin level and cell-mediated immunity in the experimental group could be due to the presence of fish oil (5%), yogurt containing LAB (24×105 cfu g^{-1}), glutamine (4%), and arginine (0.8%) in the experimental diet. Arginine has been shown to be a

semiesential amino acid for thermally injured patients. Intake of arginine after trauma and sepsis has beneficial effect by improving host immunity (Barbul, 1990).

The n-3 fatty acid in enteral diet has been shown to improve immunologic function such as the opsonic index and delayed hypersensitivity. It has been shown that fish oil feeding has protective effect on small intestinal mucosa, particularly in the region of the tips of the villi that are especially susceptible to hypoxia during sepsis because of their low oxygen concentration and high energy demand (Gerster, 1995). The improved immunological status observed in the experimental group may be attributed to the synergistic and/or additive effects of the characteristic nutrients, glutamine, fish oil, and lactobacillus microflora, although they may act through different mechanisms.

Taken together, the clinical findings suggest that the in-vivo biotransformed (malted) cereals and grain legumes combined with milk and egg and fortified with macro, micro, and other conditionally essential nutrients make suitable enteral formula for patients at hypercatabolic state such as burn injury.

Summary and Conclusion

In a prospective clinical trial undertaken at three tertiary care hospitals in India, patients of 20–50 year age group with 30–60% TBSA received the disease-specific enteral food (experimental group) as a total nutritional support. The clinical efficacy of the enteral food was compared with a control group receiving isocaloric and isonitrogenous diet from hospital kitchen. Changes in body weight, blood and urinary indices, and the immunological status were recorded periodically. The nutritional superiority of the experimental diet (DEF-BP) to that of hospital regimen was observed in terms of maintenance of body weight and muscle mass, improvement in biochemical parameters, and normalization of liver functions in the experimental group compared with the control group. Significantly improved immune status was observed in the experimental group in terms of improved cell-mediated and humoral immunity, reduced infectious episodes, rapid deep burn wound healing facilitating early grafting procedure, and percent of well-taken graft compared with the control group. A decrease in the number of hospital stays was also observed in the experimental group than in the control group.

All the patients included in the experimental group accepted the food. No incidence of diarrhea, vomiting, or ileus was reported in this group throughout the clinical trials.

In conclusion, the DEF-BP was effective to provide optimal nutritional support to the burn patients.

The next chapter includes the methodologies and results of the case studies conducted on various patients to evaluate the acceptability, tolerance, and overall efficacy of the two general-category enteral foods.

Chapter 8
Clinical Outcome of General-Category Enteral Foods

Introduction

This chapter presents the case studies relating to the clinical efficacy of the two general-category enteral foods, namely low-cost general-category enteral foods (GEF-LC) and ready-to-eat general-category enteral foods (GEF-RT) that were developed based on natural food ingredients. It was necessary to do so because usefulness of any food is established only when it serves the purpose for which it was developed and accepted by the group or individual for whom it was intended. Because these two foods were developed to meet the nutritional needs of the general-category patients, controlled clinical trials on patients suffering from specific diseases were not conducted. Instead, individual case studies were carried out on 100 patients admitted to the medical and surgical units of seven tertiary care hospitals in India. The patients received either the GEF-LC or the GEF-RT as total or partial enteral nutritional support. The overall acceptability, tolerance, and clinical outcome of the enteral foods were observed following a protocol developed for the particular patient. The general features of this protocol are presented in Appendix B.

Following are the few selected patients who received either GEF-LC or GEF-RT as total or partial enteral nutritional support.

Clinical Outcome of Low-Cost General-Category Enteral Food (GEF-LC)

Preparation of Food

The GEF-LC needs cooking prior to feeding the patient. Known quantity of GEF-LC (25–35% solid content, depending upon the need and conditions of the patients) was mixed with potable water or cow's milk and cooked at 50–60°C for 30 min in a double boiler system. Upon the completion of the hydrolysis of the complex carbohydrates by the malt enzymes present in the GEF-LC, the food was

M. Kaur, *Medical Foods from Natural Sources,*
DOI: 10.1007/978-0-387-79378-8_8,

directly heated to 100°C for 2 min and cooled to 25°C immediately. The cooked and cooled GEF-LC was then fed to the patients either orally or through a feeding tube depending upon the condition of the patient. The food was prepared freshly for each feeding under aseptic condition.

Case 1

An 8-month-old female baby was admitted to the pediatric unit of the hospital. At the time of admission, the child was in a semiconscious state with repeated spasm, continuous decerebration, high fever, diarrhea, convulsions, and melaena and was critically ill. With a working diagnosis of *shigella encephalitis* vs. *viral encephalitis* she was put on intravenous fluids, ampicillin, gentamycin, calmpose, and phenobarbitone. At the time of admission, a microcytic hypochromic picture was observed through peripheral smear, and the WBC series indicated moderate thrombocytopenia and low WBC counts. The final diagnosis was *viral encephalitis* with *hypoxic encephalopathy.*

Enteral feeding was advised to the baby, and GEF-LC was fed by bolus feeding nasogastrically (Fig. 8.1). The baby was fed every 3 h at the rate of 100 mL h^{-1} with a calorie density of 1.2 kcal mL^{-1} of GEF-LC. A total of 900 Kcal and 30 g of protein were provided per day. After 2 days of feeding, the child's general condition improved and bolus feeding was continued for a week. No incidence of diarrhea, vomiting, or ileus was reported during the feeding period. The overall health as well as the nutritional status of the baby improved gradually. Oral feeding of the same formula was initiated after a week. Enteral oral feeding with GEF- LC was continued for 18 days. By this time, the baby recovered fully and was discharged from the hospital.

At the time of discharge, blood culture showed no bacterial growth. The repeated test for peripheral smear revealed normal WBC and platelet counts. The CT scan was also normal. The baby was fully conscious with no fever, convulsions, or spasms. She was able to ingest semisolid or solid food orally. Physiotherapy and follow-up visit after 3 weeks was medically advised.

Clinical investigations on the baby on admission, at midcourse of the study, and at the time of discharge are presented in Table 8.1.

An improvement in the total blood picture with special reference to Hb, WBC, platelet count, and SGPT was observed after about 2 weeks of enteral feeding. Because the digestibility of starch and proteins, and bioavailability of vitamins and minerals are better in malt-based food compared with that in other foods (Gahlawat and Sehgal, 1994), the baby would have derived optimal nutritional support from GEL-LC, and thus facilitating recovery from a debilitating condition. There was no incidence of diarrhea, vomiting, or any other gastrointestinal discomfort during the entire period of nutritional support. While feeding orally, the baby opened her mouth voluntarily for the next serving indicating that she liked the food.

From the earlier observations, it can be concluded that GEF-LC was effective as a total enteral nutritional support to this pediatric patient suffering from *viral encephalitis* with *hypoxic encephalopathy*.

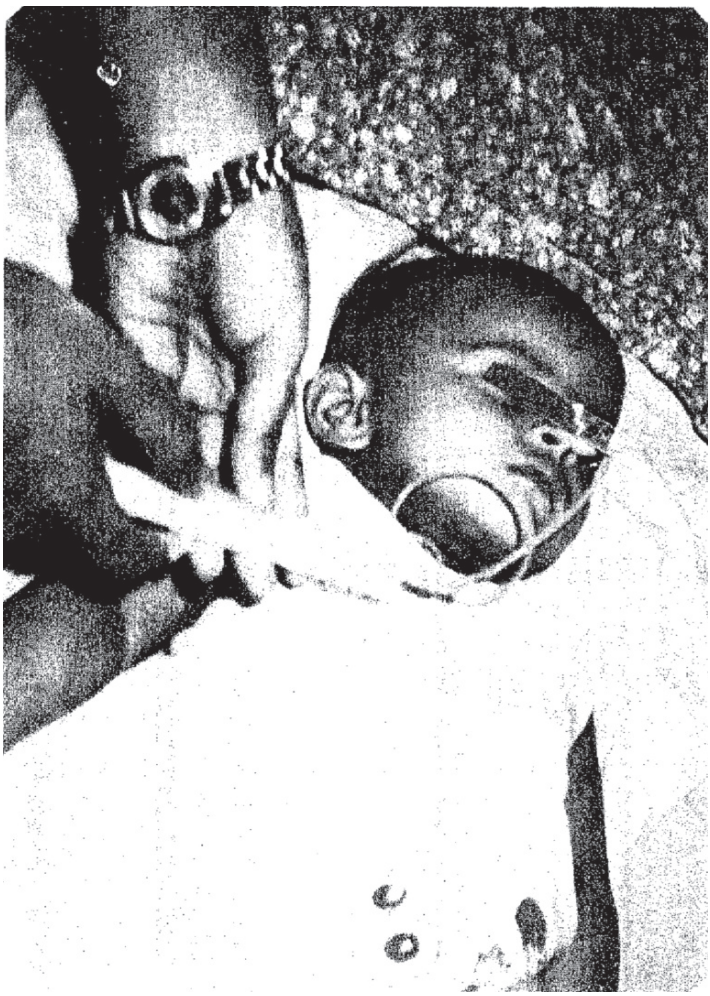


Fig. 8.1 Picture of a baby suffering from viral encephalitis with hypoxic encephalopathy receiving GEF-LC as total enteral nutritional support

Case 2

A 55-year-old male patient was admitted to the hospital with a history of chronic weight loss and painful defecation with bleeding per rectum off and on. The patient had a history of steroid dependency for psoriasis of the palms and soles. He also had a mild fissure in anus but not requiring surgery. The endoscopic examination of the upper GI tract showed a normal picture. Barium enema revealed a narrowing of the sigmoid colon, although there was no obstruction symptom. The patient underwent laparotomy, which revealed an area of the sigmoid with thickening of the wall without any growth or stricture. An end-to-end anastomosis was performed

Table 8.1 Clinical parameters of case 1

Parameters			
Blood analyses	Day 1	Day 7	Day 18
Urea (mg%)	29.0	35.0	40.0
Glucose (mg%)	35.0	50.0	60.0
Creatinine (mg%)	0.7	0.7	0.7
Total protein (g%)	6.0	6.2	7.2
Albumin (g%)	3.1	3.4	4.0
Globulin (g%)	2.9	2.8	3.2
A/*G*	1.1	1.2	1.3
Calcium (mg)	9.8	9.0	9.0
Sodium (mEq L^{-1})	129.0	130.0	131.0
Potassium (mEq L^{-1})	3.2	3.5	4.2
Chloride (mEq L^{-1})	100.0	115.0	116.0
SGPT (IU L^{-1})	130.0	78.0	65.0
Platelet count (cmm)	10,500.0	–	Adequate
WBC (cmm)	2,600.0	6,500.0	6,950.0
TLC	2,000.0	2,100.0	2,500.0
Neutrophil (%)	72.0	70.0	65.0
Lymphocytes (%)	24.0	25.0	30.0
Basophil (%)	2.0	3.0	2.0
Eosinophil (%)	2.0	1.0	2.0
Monocytes (%)	0.0	1.0	1.0
Bleeding time (min)	2.0	–	2.0
Clotting time (min)	6.0	–	5.0
ESR (first hour)	4.0	–	–
Hb (g%)	8.2	8.5	10.2
Parameters			
Urine analyses			
WBC (×1000)	1–2 pL^{-1}	nil	nil
Albumin (g%)	nil	nil	nil
Glucose (mg%)	nil	nil	nil

after resection of the portion of the sigmoid. Biopsy report revealed a *myohyperplasia of the sigmoid*.

The postoperative condition of the patient was critical with prolonged bloodstain discharge from the tube drain. A fall of hemoglobin level from 11.0 (on admission) to 7.5 g% (on 5th day) was observed, and therefore he received blood transfusion (300 mL.). Enteral nutritional support was advised, and accordingly, GEF-LC was provided as total nutritional support after 48 h of operation. Two-hundred milliliters of GEF-LC at 25% solid content was fed orally at 2-h intervals for the first 2 days. Following complete tolerance, the quantity of food was enhanced so that the patient received a total of 1,800 kcal and 70-g protein per day for 7 days. His calorie and protein density were further increased gradually to 2,670 kcal and 80 g of protein per day, respectively, till he was discharged. The patient received GEF-LC as total enteral nutritional support for a month. No adverse effect was recorded throughout the feeding period. On complete recovery, the drain and the sutures were removed.

After discharge from the hospital, he received GEF-LC as supplement for few months at home. The various clinical parameters observed during the course of the feeding trial at hospital are presented in Table 8.2.

A significant improvement in the health status of the patient was observed, measured in terms of gain in body weight (6.7%), improvement in Hb%, and normalization of WBC count and ratio between albumin and globulin (*A*/*G*) within 4 weeks of enteral feeding with GEF-LC. An improvement in the skin health was also noticed at the time of discharge. The presence of glutamine, LAB, and arginine in the GEF-LC might have helped wound healing and an overall improvement in skin health in this patient. Many investigators have reported that provision of immunomodulatory nutrients such as glutamine, LAB, arginine, fish oil, and dietary nucleotides may promote restoration of normal tissue function postoperatively, prevent the occurrence of the systemic inflammatory response syndrome, and decrease the anastomotic leaks (O'Flaherty and BouchierHayes, 1999; Waitzberg et al., 2006).

Throughout this study period, the patient readily accepted the food. The low bulking characteristics of the malt-based GEF-LC enabled him to derive optimal calories in small feedings, which otherwise would have been difficult with normal food. During a follow-up visit to the patient's home, the patient reported that he liked the taste and flavor of GEF-LC. Physicians opined that the GEF-LC was

Table 8.2 Clinical parameters of case 2

Parameters	On admission	At discharge
Body weight (kg)	42.0	45.0
Blood analyses		
Glucose (mg%)	100.0	86.0
Urea (mg%)	20.0	18.0
Creatinine (mg%)	1.6	0.9
Sodium (mEq L^{-1})	142.0	132.0
Potassium (mEq L^{-1})	4.6	4.0
Chloride (mEq L^{-1})	102.0	96.0
Total protein (g%)	6.0	6.8
Albumin (g%)	4.0	4.6
Globulin (g%)	2.0	2.8
A/*G*	2.0	1.6
ESR (min per first hour)	20.0	22.0
Hb (g%)	11.0	13.5
WBC (cmm)	12,600.0	6,400.0
Neutrophil (%)	75.0	70.0
Lymphocytes (%)	20.0	27.0
Monocytes (%)	1.0	1.0
Eosinophil (%0	3.0	2.0
Basophil (%)	1.0	0.0
Blood pressure (mm/Hg)	170/110	175/90
Prothombin time (s)	17.0	12.0
Urine analyses	Normal	Normal

effective as a complete as well as a supplemental nutritional support to this malnourished surgical patient.

Case 3

Clinical experience with an 8-year-old boy suffering from *volvulus* with *peritoneal abscess* who received GEF-LC as total enteral nutritional support showed encouraging results. Ileostomy was carried out on this patient soon after admission, and he was put on a proprietary liquid elemental enteral formula. However, he did not tolerate the elemental formula, and severe diarrhea persisted even after an adaptation period of 3 days. The elemental formula was discontinued, and nasogastric enteral nutrition through GEF-LC was initiated. Surprisingly, the patient instantly tolerated the food and normal stools were passed after 36 h. The patient received GEF-LC as tube feed for 10 days, after which oral feeding of the same was continued for two more weeks. The health of the patient improved and he was discharged from the hospital on 25th day. A few of his noticeable clinical features are presented in Table 8.3.

An increase in the body weight (7.5% of admission weight) was observed after 3 weeks of feeding with GEF-LC. The patient's hematological picture improved considerably. The overall nutritional status of the patient was also improved. A striking feature observed in this case was that the intake of this malt-based GEF-LC completely controlled the severe diarrhea, which was induced by the proprietary elemental enteral food. Lack of dietary fiber and high osmotic load (700 mOsmol kg^{-1}) of the elemental formula might have contributed to diarrhea in this patient. The presence of both soluble (2.5 g%) and insoluble (2.0 g%) dietary fiber in the form of barley β-glucan (soluble) and soy polysaccharides (insoluble) that are naturally present in the GEF-LC might have helped control diarrhea in this patient. Further, the low osmotic load of GEF-LC (246 mOsmol kg^{-1} water) might have contributed to improved tolerance of GEF-LC. Many investigators have reported the beneficial effects of dietary fiber in controlling diarrhea or constipation in enterally fed patients (Bliss et al., 2001; Topping, 2007). In addition, the bioactive lactobacilli in the form of live lactic acid bacteria might have helped control

Table 8.3 Selected clinical parameters of case 3

Parameters	On admission	At discharge
Body weight (kg)	20.0	21.5
Blood parameters		
Total protein (g%)	5.5	6.6
Albumin (g%)	2.7	3.9
Globulin (g%)	2.8	2.7
A/*G*	0.9	1.4
Hb (%)	11.0	13.0
WBC (cmm)	11,000.0	6,800.0

diarrhea in this patient. The role of bioactive lactobacilli in controlling diarrhea is well known (Patidar and Prajapati, 1997; Soomro et al., 2002; Bengmark, 2005). The patient reported that he liked the flavor of the food. GEF-LC was effective to provide optimal nutritional support to this 8-year old boy.

Case 4

Total enteral nutritional support with GEF-LC was provided to a 35-year old female patient who was admitted to the hospital with a *fecal fistula*. After a week's total nutritional support, the fistula stopped draining and also started shrinking. She was discharged form the hospital after 21 days. The patient received GEF-LC as total nutritional support throughout her hospitalization period. Home enteral nutrition was continued for 10 days as partial nutritional support. Follow-up of the case indicated that after a month the fistula had closed spontaneously and that the patient recovered fully. The patient reported that GEF-LC was palatable in porridge form as well, and the flavor was very pleasant. Because no artificial flavor was added to the GEF-LC, the pleasant flavor reported by this patient might be due to the aroma of malted cereals and grain legumes that developed during the kilning step of malting process.

Observations on clinical outcome of the aforementioned patients indicated that GEF-LC was effective to provide complete or partial nutritional support to the medical and surgical patients of various age groups.

Clinical Outcome of Ready-To-Eat General-Category Enteral Food (GEF-RT)

Preparation of Food

The GEF-RT needs to be reconstituted prior to feeding the patients. A known quantity of GEF-RT was reconstituted (25–35% solid content) with boiled and cool water under aseptic condition.

Case 5

A 42-year-old man with *chronic cirrhosis* secondary to *alcoholism* was admitted to the hospital in total unconsciousness with bilateral rigidity of the central nervous system and loss of orientation. Liver function test revealed that he was suffering from chronic alcoholic liver disease. However, the patient was maintaining a normal pulse rate (76 per min), blood pressure (120/80 mmHg), respiratory status, cardiovascular system and renal functions. He was finally diagnosed *for cirrhosis with hepatic coma* (Fig. 8.2).

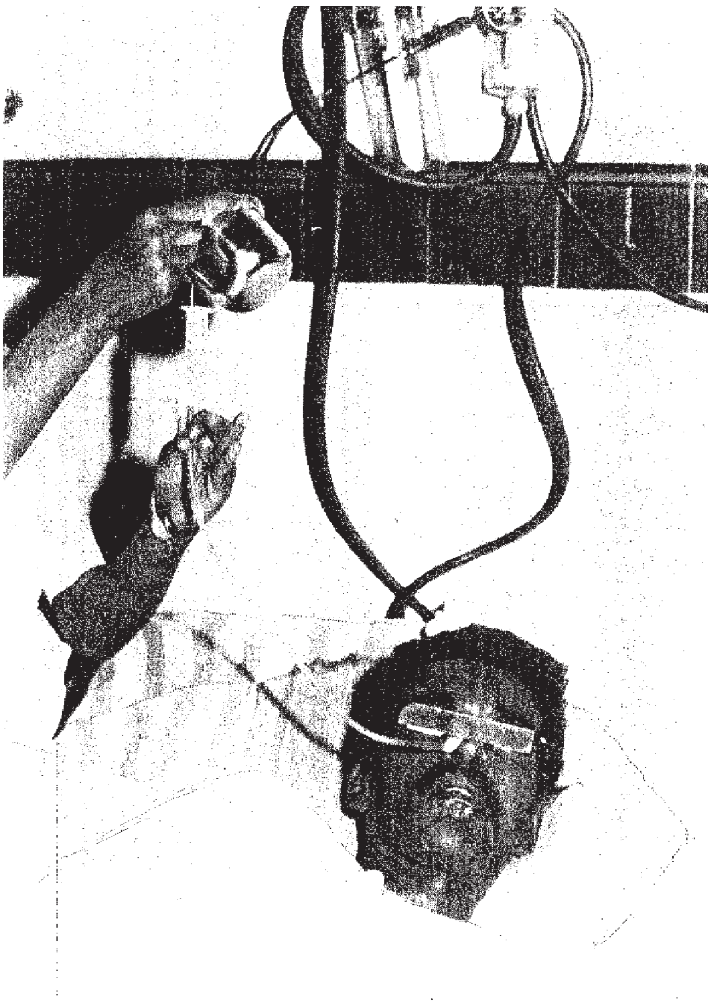


Fig. 8.2 Picture of a patient suffering from cirrhosis with hepatic coma receiving GEF-RT as nutritional support

It was difficult to assess his exact nutritional requirements because of the alterations of his body parameters such as body weight and serum sodium level due to severe ascites. The health care team advised enteral tube feeding for this patient. Based on diagnosis and the usual body weight of the patient, the optimal calorie and protein requirements were calculated to be 2,500 kcal and about 50-g protein per day. Accordingly, total enteral nutritional support was provided to him with GEF-RT nasogastrically throughout the hospitalization period. The overall health status of the patient improved and showed stable vital signs and sensorium. The patient was discharged from the hospital on 55th day in semiconscious state. Home

enteral nutrition support continued for another 45 days. The food was accepted and tolerated by the patient without diarrhea, vomiting, or any other gastrointestinal disturbances. The data on the clinical investigations carried out during the feeding trial are presented in Table 8.4.

At the time of discharge, an increase in body weight (6.2%), mid arm circumference (11%), and tricep skin fold thickness (29%) was recorded. Biochemical investigations with special reference to A/G, SGOT, SGPT, and complete hemogram showed that the values were within the normal range. Like most of the cirrhotic patients, this patient was also malnourished and wasted. The patient was probably benefited by the enteral alimentation with GEF-RT, which contained vegetable proteins, enhanced amount of BCAA, reduced amount of AAA, live probiotics (LAB), soluble dietary fiber, taurine, carnitine, and other essential macro and micronutrients.

Clinical studies have shown that patients with cirrhosis of liver with chronic portal–systemic encephalopathy tolerated vegetable proteins better than animal

Table 8.4 Clinical parameters of case 5

	Days of investigations						
Parameters in Blood	1	7	15	22	36	50	65
Glucose (mg%)	312.0	166.0	150.0	134.0	110.0	100.0	95.0
Urea (mg%)	28.0	28.0	28.0	26.0	23.0	19.0	18.0
Creatinine (mg%)	1.4	1.1	1.0	1.0	1.1	0.9	0.9
Sodium (mEq/L^{-1})	132.0	133.0	132.0	133.0	135.0	136.0	135.0
Potassium (mEq/L^{-1})	4.1	4.2	4.0	4.2	4.5	5.0	4.8
Chloride (mEq/L^{-1})	100.0	100.0	98.0	98.0	97.0	97.0	97.0
Total protein (g%)	6.2	6.1	6.0	6.2	6.4	6.5	6.7
Albumin (g%)	3.0	3.0	3.2	3.4	3.8	3.9	3.9
Globulin (g%)	3.2	3.1	2.8	2.8	2.6	2.6	2.8
A/G	0.9	1.0	1.1	1.2	1.4	1.5	1.4
Bilirubin (mg%)	0.3	0.3	0.3	0.2	0.1	0.1	0.1
SGOT (IU/L^{-1})	118.0	110.0	98.0	85.0	40.0	35.0	35.0
SGPT (IU/L^{-1})	65.0	64.0	60.0	50.0	35.0	19.0	19.0
Magnesium (mg%)	2.0	1.8	1.9	1.8	1.7	1.8	1.8
Phosphate (mg%)	2.8	2.6	2.7	2.5	2.6	2.6	2.7
Hb (%)	10.0	10.2	10.4	10.6	11.0	11.5	12.2
WBC (cmm^3)	5,700.0	5,800.0	5,800.0	6,800.0	7,500.0	9,500.0	9,500
Neutrophill (%)	58.0	58.0	58.0	61.0	62.0	64.0	64.0
Lymphocytes (%)	30.0	31.0	32.0	32.0	33.0	34.0	35.0
Monocytes (%)	5.0	5.0	4.0	3.0	2.0	2.0	1.0
Eosinophil (%)	7.0	6.0	4.0	3.0	2.0	2.0	0.0
Basophil (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Parameters in urine							
Glucose (mg%)	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Pus cells	10–15	8–9	8.0	5–6	4–6	3–4	2–3
RBC	2–3	0.0	0.0	0.0	0.0	0.0	0.0
Albumin (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Calcium	+	+	+	+	–	–	–

protein, and encephalopathy index was lower after vegetable-protein diet than animal-protein diet (Javaglia et al., 1992; Bianchi et al., 1993; Kircheis and Haussinger, 2002). The favorable therapeutic effect of vegetable protein diets on nitrogen metabolism can mainly be accounted for the increased intake of dietary fibers and increased incorporation and elimination of nitrogen in fecal bacteria (Javaglia et al., 1992; Bianchi et al., 1993). Studies also have shown that BCAA-supplemented diet may be useful for treatment of impaired protein metabolism frequently observed in cirrhotic patients. Further, it was reported that BCAA might contribute to maintaining a positive nitrogen balance and minimize muscle wasting in patients with cirrhosis (Javaglia et al., 1992; Marchesini et al., 2003).

The presence of live LAB in the GEF-RT might have also benefited the patient. Ferency (1996) reported that one of the treatments of hepatic encephalopathy in patients with cirrhosis of liver could be based on reducing ammoniagenic factors in the intestinal tract, and promoting the elimination of nitrogenous waste from the body. He also reported the role of LAB in reducing the intestinal pH and thereby facilitating the removal of ammonia. The live LAB present in the GEF-RT probably played a role in modification of colonic pH in this patient and thereby facilitated the removal of ammonia. However, more clinical studies in this line are necessary to confirm the role of LAB in removal of ammonia.

Hepatic failure is sill a challenging problem where nutritional support plays a key role. In this direction, this case study showed that an enteral food based on vegetable proteins containing BCAA and live lactic acid bacteria may be useful in providing optimal nutritional support to cirrhotic patient with hepatic encephalopathy.

Case 6

It was necessary to admit a 40-year-old man when he came as an outpatient to the hospital with a complaint of severe dysphagia. His histopathological diagnostic test revealed a frank *squamous cell carcinoma* and obstruction of the esophageal lumen causing dysphagia. His nutritional status as assessed by anthropometric, hematological, and biochemical parameters indicated that he was suffering from *cancer cachexia*.

A gradual yet aggressive nutritional support to the patient was required to improve his nutritional status that would enable him to withstand proper oncologic therapy. Because he had severe dysphagia, a nasogastric feeding tube was inserted through the malignant stricture under fluoroscopic guidance, and enteral tube feeding with GEF-RT was initiated. The diet (30-g GEF-RT + 5-g sugar) was reconstituted in 200-mL homogenized milk (3.25% milk fat) and was delivered to the patient as bolus feed at 2-h intervals to attain a goal of 2,800 kcal and 100-g protein per day. The feeding was increased slowly as tolerated by the patient, and the goal for calorie and protein requirements was achieved within 5 days. Strict observation was made regarding onset of refeeding syndrome, diarrhea, vomiting, or ileus. Feeding continued for 2 months after which a considerable improvement in his

health status was recorded and he was certified fit for undergoing surgery. Study was terminated at this stage. Food was well accepted and tolerated by the patient. The nutritional status of the patient recorded during the study period is presented in Table 8.5.

The depletion of body fat and low serum albumin content of the patient observed at the time of admission may be due to inadequate food intake because of severe dysphagia caused by the primary disease – *carcinoma esophagus.* After prolonged enteral feeding (40 days) an increase in body weight (5%), TSF (28%), MAC (10%), MAMC (18%), total protein (1.3%), and Hb (1.3%) of admission was observed. During the feeding period, symptoms such as regurgitation, nausea, vomiting, and fluid overload were not reported. Blood sugar level, renal function test, liver function test, and serum electrolytes remained within the normal range. Further, a significant improvement in serum albumin level was recorded toward the end of the study. The absence of diarrhea or vomiting could be due to the favorable osmolality (350–400 mOsmol kg^{-1}) of GEF-RT. The presence of soluble and insoluble fiber, lactic acid bacteria, and predigested form of intact nutrients derived from GEF-RT might also have helped the patient tolerate the food. The improvement in the body weight, hemoglobin level, and skeletal and visceral protein store and repletion of adipose tissues indicated that the GEF-RT provided optimal nutritional support to this patient with *cancer cachexia.*

From these case studies, it can be inferred that the general-category enteral foods (low-cost as well as ready-to-eat formulae) were suitable for providing partial or total nutritional support to patients of wide range of physiological conditions. Home enteral nutritional support with these formulae was also carried out successfully. The easily digestible carbohydrates, proteins of vegetable origin, lipids containing suitable proportions of PUFA, and probiotics present in the foods contributed to providing optimal nutritional support to the patients.

The low-cost enteral food (GEF-LC) should be suitable for patients belonging to very low socioeconomic strata especially from developing or underdeveloped countries whose daily nutritional requirement can be met at about less than 3 US$ a day. On the other hand, the patients belonging to middle and high socioeconomic strata could afford the ready-to-eat enteral food (GEF-RT), which will cost about 5–8 US$ per patient per day. Further, the patients who consume vegetarian diet only (lactovegetarian) will readily accept these two formulae.

Table 8.5 Nutritional status of case 6

Days	Wt (kg)	BMI (kg m^{-2})	TSF (mm)	MAC (cm)	MAMC (cm)	Hb (g%)	Total protein (g%)	Albumin (g%)	*A/G*
1	42.0	16.0	7.3	17.3	16.0	11.7	4.9	2.5	1.0
20	43.6	16.6	7.7	17.6	16.3	12.3	5.6	3.0	1.1
40	44.2	16.8	9.3	19.0	18.9	13.0	6.2	3.4	1.2

Summary and Conclusion

The clinical outcome studies of the general-category enteral foods were evaluated on a large number of patients admitted to the medical and surgical units of seven tertiary care hospitals of India. The relevant clinical data were collected as per the protocol developed to suit the physiological conditions and nutritional demands of the patients.

Total enteral nutritional support through the low-cost and GEF-RT was provided to 100 patients of general medicine and surgery including those in pre- and postoperative state. The foods proved to be effective in providing optimal nutritional support to the patients as assessed by improvement in nitrogen balance, blood parameters, reduction in hospital stay, and early spontaneous closure of draining fistula among others.

In conclusion, it can be stated that malted grain- and milk-based enteral foods fortified with other nutrients and probiotics can provide optimal nutritional support to the general-category patients at an affordable price.

Chapter 9
General Summary and Conclusion

Summary and Conclusion

Most of the hospitalized patients are malnourished. Optimal nutritional support to the patients helps speedy recovery, facilitates effectiveness of medical treatment, brings down the hospitalization expenses, and reduces the morbidity and mortality rate. Hence, provision of optimal nutritional support to the patients either enterally (feeding via gut) or parenterally (infusing sterile nutrients via vein, thereby bypassing the gut) is a part of medical treatment in most of the health care facilities. Although parenteral nutrition support could be lifesaving in certain situations, its prolonged use may not be desirable, because it fails to maintain gut physiology and functions. On the other hand, because enteral nutrition support maintains gut integrity and functions, patients having a fully or partially functional gut but unable to nourish themselves optimally are encouraged to receive enteral nutrition support. Patients suffering from cancer, renal failure, liver failure, comatose, sepsis, burn injury, and those admitted to the intensive care units among others often require enteral nutrition support. Enteral nutrition support is provided through specially designed formulae known as enteral foods. Enteral foods are a class of liquid medical foods fed to the patients either orally, or through a feeding tube under medical supervision. The importance of enteral nutrition support to the patient in hospital and home is well recognized, and an array of general-cagory (standard) and disease-specific (to meet the nutritional demands of various disease conditions) enteral foods has been developed and marketed globally.

Most of the enteral foods currently marketed are prepared by blending defined- or chemically-defined food ingredients such as maltodextrin, sucrose, glucose, peptides, amino acids, and glycerides. They are usually priced high and lack the natural stimulants and protectants that are present in normal foods. Further, to improve the acceptability and to mask the chemical nature of the ingredients, these foods are often artificially flavored and excessively sweetened. To overcome these drawbacks associated with defined ingredient-based enteral foods, a need was felt to develop enteral foods akin to natural foods that could be afforded by the majority of the needy patients. With these objectives, research work on development of medical foods (enteral foods) based on natural ingredients was undertaken, which is presented in this book.

M. Kaur, *Medical Foods from Natural Sources,*
DOI: 10.1007/978-0-387-79378-8_9, © Springer Science+Business Media, LLC 2009

The nutritional and the textural characteristics of cereal malts were gainfully exploited to develop two general-cagory enteral foods and one disease-specific enteral food. Rice malt, barley malt, finger millet malt, mung bean malt, popped grain amaranth, defatted soy flour, milk, yogurt, egg, soy oil, and fish oil were used as the major ingredients of the enteral foods.

Because malting of rice for the preparation of food is a new concept, malting conditions for rice were standardized. Rice (rough rice or paddy) was steeped in water for 24 h and germinated for 72 h. The sprouts were sun-dried or mechanically dried, derooted, and kilned by contact heat in a grain roaster at 60–70°C to prepare the rice malt. Malted rice was dehusked in a rubber roll sheller, and the brown rice malt was moist-conditioned and milled in a roller flour mill to obtain fiber-regulated and enzyme-rich rice malt flour. Food grade barley malt procured from a commercial malt house was also moist-conditioned and milled in roller flour mill to prepare fiber-regulated, enzyme-rich barley malt flour suitable for enteral foods. The yields of rice and barley malt flours were 72% and 69%, respectively. The malt flours were rich sources of amylases, and their dietary fiber contents were about 8%. Flow charts for milling of malted rice and barley were also developed. Malt flour from 2-day germinated finger millet and 1-day germinated mung bean was prepared following the standard methods. Popped amaranth grain was prepared by HTST treatment.

Rice malt, barley malt, millet malt, mung bean malt, defatted and toasted soy flour, whole milk powder, and soy oil were blended in suitable proportion and the blend was fortified with vitamins, minerals, lactic acid bacteria, taurine, carnitine, and L-glutamine to prepare a low-cost general-cagory enteral food (GEF-LC). The malted cereals, malted mung bean, defatted soy flour, and soy oil were mixed with potable water and cow's milk, and the slurry was cooked under controlled temperature and time to facilitate maximum hydrolysis of starch by the malt enzymes. The mash was cooled to 25°C, and to that yogurt containing live lactic acid bacteria was added. The slurry was homogenized and spray dried to prepare ready-to-eat general-cagory enteral food (GEF-RT). A disease-specific enteral food suitable for meeting the extended nutritional demand of burn patients (DEF-BP) was also prepared similar to GEF-RT, where fresh hen's egg, popped grain amaranth, and fish oil formed additional ingredients.

The foods contained 16–21-g proteins and 410–460 kcal per 100 g in dry form. A liter of food at 25–30% solid content provides about 1,100–1,600 kcal, 40–65-g proteins, and 12–13-g dietary fibers. The osmolality and the renal solute load of the foods were in the range of 246–500 mOsmol kg^{-1}, and 195–219 mOsmol/L, respectively. The foods exhibited free-flowing property at 20–35% solid contents through 6–18 FG tubes under gravity. The carbohydrates of the foods were predigested, and the molecular distribution of the carbohydrate fractions was in the range of 5–15 dextrose equivalents (DE). The food proteins were of balanced essential amino acid profile and the proteins were largely of intact form. Nearly 65% of the lipids were unsaturated fatty acids, maintaining desirable ratios between SFA/MUFA/PUFA as well as between n-6 and n-3 PUFA. The growth-promoting quality of the food protein was determined by rat feeding trials, and the PER values for GEF-LC, GEF-RT, and DEF-BP were 2.9, 2.7, and 3.4, respectively. The biological value and the digestibility

of the food proteins were also high, and the NPU was over 75% indicating that the proteins of the enteral foods were of balanced amino acid profile. The histopathological examination of the liver and other vital organs of the animals fed on enteral foods showed normal picture without any visible fatty infiltration and malformation. The results of the animal experimentation revealed that the food proteins were of good quality and the foods were free from toxic components.

The foods were microbiologically safe. Lactic acid bacteria contributed to the major proportion of microbial load. The pesticide residue contents of the enteral foods were well within the permissible limits. The foods met the international criteria laid down for medical foods.

The shelf-life of the low-cost general-cagory enteral food was about 90 days in polypropylene pouches at ambient storage conditions, whereas the ready-to-eat general-cagory and the disease-specific enteral foods packed in aluminum foil laminate remained acceptable for about 300 days at ambient storage conditions. Vacuum packing enhanced the shelf-life of foods by another 90 days.

The clinical efficacy of the general-cagory enteral foods was established by providing total or partial nutritional support to 100 patients of pediatric to geriatric age group, who were admitted to the medical, surgical, and intensive care units of seven tertiary care hospitals of India. The foods were well accepted and tolerated by all the patients. Improvement in body weight and biochemical indices as well as reduction in hospital stay was observed in most of the cases.

Preliminary clinical trials on the enteral foods for burn patients (DEF-BP) were conducted on six patients with 50–80% total body surface burns (TBSA) admitted to two tertiary care hospitals of India. Controlled clinical trial was conducted at another tertiary care hospital of India on 40 patients with 30–80% TBSA, who were divided between control and experimental groups. The patients of the experimental group received total nutritional support from DEF-BP alone, whereas the control group received the isocaloric and isonitrogenous diet from the hospital kitchen designed for burn patients. Significant nutritional benefits were observed in the experimental group compared with that in the control group in terms of improvement in body weight, biochemical indices, and humoral and cell-mediated immune responses. Further, healing of deep burn wound was rapid in the patients of the experimental group and percentage of well-taken graft was more in this group than in the control group. Furthermore, the duration of hospital stay was significantly lesser in the experimental group than in the control group. The DEF-BP was well accepted and tolerated by all the patients.

The study revealed that malted cereals and legumes along with popped grain amaranth, milk, egg, and soy and fish oils can be suitably processed to prepare nutritionally balanced and texturally compatible general-cagory and disease-specific enteral foods. In sum, the following conclusions can be drawn:

1. Rice could be malted similar to other cereals. Fiber-regulated, enzyme-rich malt flour suitable for medical foods could be prepared from malted rice. Rice malting could be altogether a new avenue for the diversified uses and value addition to rice.

2. The malted cereals and grain legumes could be gainfully utilized for development of nutritionally balanced and cost-effective enteral foods.
3. The coagulation characteristics of the egg protein upon heating could be altered by suitably processing fresh egg with malted cereals and milk, which enables to prepare ready-to-eat, egg-based medical foods of improved textural and nutritional qualities suitable for tube feeding.
4. The nutrient composition and the physicochemical characteristics of the natural ingredients-based enteral foods are comparable with that of proprietary enteral foods based on chemically defined ingredients. In fact, the acceptability and tolerance of the natural ingredient-based enteral foods are better than that of the defined ingredients-based proprietary enteral foods.
5. It is possible to enrich the enteral foods with probiotics from a natural food source such as yogurt, and its lactic acid bacterial flora survives during spray drying. These foods are rich source of lactic acids and are microbiologically safe.
6. Enteral foods based on malted cereals, milk, and egg possess good growth-promoting qualities. Their proteins are of high biological values and are comparable with that of defined ingredients-based enteral foods. In addition, their proteins are of intact form and free from natural or other toxins.
7. The low-cost general-cagory enteral food could be packed in low-priced flexible packaging materials such as polypropylene and polyethylene. The spray-dried ready-to-eat enteral foods need to be packed in 100% moisture barrier packaging materials such as aluminum foil laminates, glass bottles, or metallic tins. The lactic acid bacteria added to the foods survived during the specified storage period.
8. The disease-specific enteral foods, based on cereal malts, milk, and egg that was developed to meet the extended nutritional demands of the patients at hypercataboilc state, are suitable to provide optimal nutrition support to burn patients.
9. The malted cereal-based enteral foods are suitable to provide total or partial nutritional support to a wide range of patients of general medicine and surgery.
10. The probiotics such as lactic acid bacteria and bifiodo bacteria among others help tolerate the milk-based enteral foods.
11. The cost of malted cereal-based enteral foods is lower than that of the defined- or chemically-defined ingredients-based enteral foods.

Of a particular note, the low-cost general-cagory enteral food (LCEF) would offer cost-effective enteral nutrition support to a wide range of patients in developing and underdeveloped countries.

Implications for the Scientists and Healthcare Professionals

This study offers the following implications for the scientists and health care professionals.

The first implication is that there is a need to develop enteral foods from natural sources that are more physiological and cost-effective than the defined ingredients-based

enteral foods. Most of the enteral foods currently available contain maltodextrin as source of carbohydrates, hydrolyzed proteins, peptides, or amino acids as sources of proteins, and soy polysaccharides as source of dietary fibers. Because these ingredients are defined- or chemically defined ingredients, besides being costly, they are unpalatable, need artificial flavorings, and lack the natural stimulants and protectants that are present in natural foods.

However, the findings of the book show that it is possible to prepare enzyme-rich and fiber-regulated flour from malted cereals with a carbohydrate profile similar to that of maltodextrin. Further, bioavailability of proteins, certain vitamins, and minerals is increased during malting. Furthermore, malted cereals and grain legumes possess pleasant flavor, provide dietary fiber, and contain other protective components; hence, they are suitable for incorporating in enteral food formulations. The technology described in this book to prepare the low-cost general-cagory enteral food (GEF-LC) could be effectively employed in small-scale business environment. This has special implication for the scientists and health care professionals in developing and underdeveloped countries such as India, China, Pakistan, Bangladesh, South America, and Africa, among others. These countries could exploit this inexpensive technology to prepare low-cost enteral foods based on their locally available cereals and grain legumes. Thus, it will be possible to provide optimal nutritional support to majority of the patients belonging to lower socioeconomic strata at an affordable cost. This book shows how to integrate the simple food processing technologies for value addition to natural food ingredients.

The second implication of the study is that dietitians, nutritionists, and other health care professionals may like to exploit the benefits of foods rich in probiotic microflora while providing the enteral nutritional support to patients at hospital and home. Clinical outcome of this study shows that majority of the patients admitted to hospital or treated at home could tolerate the GEF-LC, GEF-RT, and DEF-BP where milk is one of the major ingredients. Even those patients with mild to moderate degree of lactose intolerance may be able to tolerate milk and milk products such as yogurt, cottage cheese, sour cream among others that contain active bacterial culture such as *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, lactic acid bacteria, and bifido bacteria. Thus, it is not necessary to consider the use of the lactose-free formulae by default when providing nutritional support to patients. However, careful assessment and individualization are necessary for such an effort. In general, whenever possible, everybody should be encouraged to derive the natural health benefits of milk and milk products. In recent years, studies have confirmed the combined beneficial effect of probiotics and plant fiber (together known as synbiotics) in managing lactose intolerance and many other gastrointestinal diseases. The present study also supports the beneficial effect of synbiotics from natural sources (e.g., yogurt and dietary fiber) in nutrition support to patients. Therefore, another implication for the health-care professionals is to provide synbiotics from natural food sources in the diet of enterally fed patients.

Finally, one of the findings of this study, which is consistent with other studies, is that enteral foods containing vegetable protein and enriched with branched chain amino acids are effective in nutritional support to the patients with cirrhosis of liver

with encephalopathy. Because mung bean is a rich source of branched chain amino acids, yet another implication for dietitians and nutritionists is that they may consider incorporating foods prepared from mung bean in the diet of patients with cirrhosis of liver with encephalopathy. Results of a case study included in this book (Chap. 8) show that an enteral food (GEF-RT) based on malted cereals, malted mung bean, yogurt, and milk was effective in providing optimal nutrition support to a patient suffering from cirrhosis of liver with encephalopathy. This has special implication for those providing nutrition support to such patients in developing and underdeveloped countries, where branched chain amino acid-enriched proprietary enteral formulae may be beyond the reach of majority of the patients.

I hope that this book gives food for thought to scientists, food technologists, practicing nutritionists, dietitians, and other health-care professionals about how to exploit the benefits of natural food while attempting to provide the optimal nutritional support to the patients at an affordable cost.

Extension to the Study

This study may be further extended in the following directions.

Because nutritional needs of patients suffering from various disease conditions are different, there are scopes for developing a wide range of disease-specific enteral foods from natural food ingredients. In the present study, three (two general-cagory and one disease-specific) enteral foods based on natural food sources have been developed. These three foods are in powder form as they contain added glutamine, which is unstable in liquid medium. This study can further be extended to develop liquid form of enteral foods from natural sources. Second, following repeated attempts, the coagulation characteristic of hen's egg upon heating was reversed to free-flowing characteristics, and thus enabling them to incorporate in the disease-specific enteral food for burn patients. However, we do not have the answer for *how did it happen*? Presumably, malt enzymes present in the cereal base of the enteral foods played a role in modifying the coagulation characteristics of heat-treated egg. Therefore, further investigations are encouraged to determine the actions of and interactions between the various bioactive food components such as malt enzymes, dietary fiber, live lactic acid bacteria, and fresh hen's egg that enables the egg protein to remain free flowing after heat treatment.

Next, the effectiveness of the natural ingredients-based general-cagory enteral foods has been evaluated following a case-study method. Controlled clinical outcome studies of the natural ingredients-based and defined ingredients-based general-cagory enteral foods may be conducted to find out the differences between these two types of enteral foods. However, while conducting controlled clinical trials, it is important to select the patient sample that represents the general-cagory patients' population.

Finally, besides rice, barley, finger millet, grain amaranth, soybean, and mung bean, there are other natural food sources that deserve feasibility studies regarding

their suitability to incorporate in the development of medical foods. Conducting such feasibility studies is particularly encouraged for the researcher in developing and underdeveloped countries so that they can utilize their locally available low-cost natural food sources for the development of medical and other specialty foods.

To conclude, I sincerely believe that the readers of this book will be motivated to do further research toward development of cost-effective *medical foods from natural sources*.

Appendices A-C

Appendix A

Nutritional Profile of Selected Enteral Foods

Product name and source	Jevity 1.0 Ross[1]	Pulmocare Novartis[2]	Isosource HN Novartis[2]	Nutren fibre Nestle[3]
Form	Liquid	Liquid	Liquid	Liquid
Kcal mL^{-1}	1.06	1.5	1.2	1.0
Prot. (% of Cal)	16.7	16.7	18.0	16.0
Carb. (% of Cal)	54.3	28.2	51.0	50.0
Fat (% of Cal)	29.0	55.1	31.0	34.0
Protein (g L^{-1})	44.3	62.6	53.0	40.0
Carb. (g L^{-1})	154.7	105.7	151.0	130.0
Fat (g L^{-1})	34.7	93.3	42	38
Cal:N	150:1	150:1	143:1	156:1
Sodium mg(mEq)	930 (40.4)	1,310 (13.5)	1,120 (49.2)	870
Potassium mg(mEq)	1,570 (40.20)	1,960 (11.9)	1,800 (46.0)	1,240
Osmolality (mOsmol kg^{-1})	300	475	435	330 (unflavoured) 410 (vanilla)
Renal solute load (mOsmol L^{-1})	370	512	–	332
Fibre (g/L)	14.4	–	–	14.0
Prot. source	Sodium/ calcium caseinate	Sodium/ calcium caseinate	Sodium caseinate Soy protein isolate	Calcium/ potassium caseinate
Carb. source	Sugar, corn Maltodextrin	Corn syrup Maltodextrin	Maltodextrin Corn syrup solid	
Fat Source	Canola Oil Corn Oil Safflower oil MCT	Canola oil MCT	Canola oil MCT	

(continued)

[1]www.ross.com (product hand book)
[2]Novartis Medical Nutrition, Pocket Guide, 2005
[3]Nestle Nutrition, Pocket Guide, 2007

(continued)

Product name and source	Jevity 1.0 Ross[1]	Pulmocare Novartis[2]	Isosource HN Novartis[2]	Nutren fibre Nestle[3]
Flavor		Vanilla Strawberry	–	Vanilla
Other features	Lactose free Gluten free Kosher	Lactose free Gluten free Kosher Low residue	Lactose free Gluten free Kosher	Lactose free Gluten free Kosher
Recommended	Long-term tube feeding	COPD, cystic fibrosis	Long-term tube feeding	Tube/oral feeding, weight mgmt.
		Respiratory failure	–	Diarrhea/ Consti-pation
ML to meet 100% RDI for vitamin/ mineral	1,321	947	–	–

Product name and source	Glucerna Ross[1]	Promote w/fiber Novartis[2]	Peptamen Novartis[2]	Nutrihep Nestle[3]
Kcal mL^{-1}	1.0	1.0	1.0	1.5
Prot. (% of Cal)	16.7	25.0	16.0	11.0
Carb. (% of Cal)	34.3	50.0	49.0	77.0
Fat (% of Cal)	49.0	25.0	35.0	12.0
Prot. (g L^{-1})	41.8	62.5	40.0	40.0
Carb. (g L^{-1})	95.6	138.3	130.0	290.0
Fat (g L^{-1})	54.4	28.2	39.0	21.2
Cal:N	150:1	100:1	156:1	234:1
Sod. mg(mEq)	930(–)	1,300(–)	560(–)	160(–)
Pot. mg(mEq)	1,570(–)	2,100(–)	1,500(–)	1,320(–)
Osmolality (mOs-mol kg^{-1})	355	380	270 (unflavor) 380 (Vanilla)	790
Renal solute Load (mOs-mol L^{-1})	360.0	499.0	319.0	301.0
Fiber (g L^{-1})	14.4	14.4	–	–
Prot. source	Sodium/ calcium caseinate	Sodium/calcium caseinate	Hydrolyzed whey	Crytalline L-amino acids
		Soy protein isolate	Protein	Whey protein concen-trate
Carb. Source	Corn Maltodextrin	Corn Maltodextrin	Maltodextrin Corn starch	Maltodextrin Modified corn starch
Fat Source	Safflower oil, canola oil	Safflower oil, soy oil MCT	Soy oil MCT	Canola oil Corn oil MCT

(continued)

(continued)

Product name and source	Glucerna Ross[1]	Promote w/fiber Novartis[2]	Peptamen Novartis[2]	Nutrihep Nestle[3]
Flavor	Unflavored Vanilla	Vanilla	Unflavored Vanilla	–
Other features	Lactose free Gluten free Kosher	Lactose free Gluten free Kosher	Lactose free Gluten free Elemental	Lactose free Gluten free Low residue Kosher High BCAA Low AAA
Recommended Usage	Type I &II Diabetes mellitus	Wound Healing Pressure Ulcer, PEM	Transition from TPN Pancreatitis Croh's Disease	Tube/oral feeding, hepatic disease
ML to meet 100% RDI for vitamin/ mineral	1,420	–	–	–

Product name and source	Resource Diabetic Novartis[2]	Impact Novartis[2]	Novasource Renal Novartis[2]	Vivonexplus Novartis[2]
Form	Liquid	Liquid	Liquid	Liquid
Kcal mL^{-1}	1.0	1.0	2.0	1.0
Prot. (% of Cal)	24.0	22.0	15.0	18.0
Carb. (% of Cal)	36.0	53.0	40.0	76.0
Fat (% of Cal)	40.0	25.0	45.0	6.0
Prot. (g L^{-1})	64.0	56.0	74.0	45.0
Carb. (g L^{-1})	95.0	130.0	200.0	190.0
Fat (g L^{-1})	47.0	28.0	100.0	6.7
Cal:N	104:1	91:1	164:1	140:1
Sod. mg(mEq)	960(42)	1,100(48)	900(39)	610(27)
Pot. mg(mEq)	1,400(36)	1,760(45)	810(21)	1,060(27)
Osmolality (mOsmol kg^{-1})	–	375	700	650
Renal solute load (mOsmol L^{-1})	–	–	–	–
Fiber (g L^{-1})	12.0	–	–	–
Prot. source	Sodium/ calcium caseinate Soy protein isolate	Sodium/ calcium caseinate L-arginine	Sodium/ calcium caseinate L-arginine	Free amino acid
Carb. source	Corn syrup solids Fructose	Hydrolized corn starch	Corn syrup Fructose	Maltodextrin Modified corn starch
Fat Source	Sunflower oil, soybean oil	Palm kernel oil, sunflower oil	Sunflower oil Corn oil MCT	Soybean oil

(continued)

(continued)

Product name and source	Resource Diabetic Novartis[2]	Impact Novartis[2]	Novasource Renal Novartis[2]	Vivonexplus Novartis[2]
Flavor	–	–	Mild Vanilla	–
Other features	Lactose free Gluten free	Lactose free Gluten free Low residue	Lactose free Gluten free Low residue	Lactose free Gluten free Low residue
Recommended Usage	Type I &II diabetes mellitus Increased protein requirement	Trauma Major surgery Burn injury	Acute/chronic renal failure Fluid restrictions	Bowel rejections Croh's Disease Fistula Pancreatic disorder
ML to meet 100% RDI for vitamin/mineral	–	–	–	–

Product name and source	Similac Ross[1]	Pediasure Ross[1]	Vivonex/pedia. Novartis[2]	Nutren Jr. Nestle[3]
Form	Liquid	Liquid	Powder	Liquid
Kcal mL^{-1}	0.7	1.0	0.8	1.0
Prot. (% of Cal)	11.0	12.0	12.0	12.0
Carb. (% of Cal)	41.0	35.0	63.0	44.0
Fat (% of Cal)	48.0	53.0	25.0	44.0
Prot. (g L^{-1})	18.6	30.0	24.0	30.0
Carb. (g L^{-1})	69.0	133.0	130.0	110.0
Fat (g L^{-1})	37.4	40.0	24.0	50.0
Cal:N	–	–	227:1	208:1
Sod. mg(mEq)	298(13)	380(17)	–	460
Pot. mg(mEq)	798(20)	1,370(34)	–	1,320
Osmolality (mOsmol kg^{-1})	370.0	335.0	360.0	350.0
Renal solute load (mOsmol L^{-1})	171.3	277.0	–	256.0
Fiber (g L^{-1})	–	–	–	–
Prot. Source	Casein hydrolysate L-cystine L-tyrosine l-tryptophan	Milk protein Concentrate	Free amino acid	Milk protein Concentrate Whey protein concentrate
Carb. source	Sugar, modified corn starch	Corn Maltodextrin, sucrose	Maltodextrin Modified corn starch	Maltodextrin Sucrose

(continued)

(continued)

Product name and source	Similac Ross[1]	Pediasure Ross[1]	Vivonex/pedia. Novartis[2]	Nutren Jr. Nestle[3]
Fat Source	Sunflower oil Soy oil	Safflower oil MCT	Soy oil MCT	Canola oil MCT
Flavor	–	Vanilla	–	–
Other features	Hypoallergic Lactose free Corn free	Milk-based Lactose-free Gluten-free Kosher	Lactose-free Gluten-free Low residue	Lactose free Gluten free Low residue Kosher
Recommended usage	Infant with colic symptoms, children with food allergies, sensitivity to Intact protein	For 1–13-yr old children, tube or oral feeding, complete/ supplemental nutrition support	For 1–9-yr old children, TPN transitional feeding, trauma, surgery, Crohn's diseases, GI disorder related to AIDS, short bowel syndrome	For 1–9-yr old children, tube or oral, growth failure

Appendix B

Protocol for Clinical Trials on Enteral Foods

A. Personal Information

1. Name:
2. Sex: M/F
3. Age:
4. Occupation:
5. Hospital No:
6. Address:
7. Telephone No.

B. Hospital Records

1. Date of admission:
2. Date of discharge
3. *Details of burn injury:
   (a) Number of days after burn injury:
   (b) Cause: accidental/suicidal/homicidal
   (c) Mode of injury: flame/scalds/electrical/chemical
   (d) Total body surface area burns (percnt;):
   i Superficial ii Deep dermal iii Full thickness
4. Energy requirements: kcal day $^{-1}$
5. Protein requirements: g day $^{-1}$

C. Past medical history

1. Normal: Yes/No
2. Diabetes: Yes/No
3. Hypertension: Yes/No
4. CHD/IHD/PVD
5. Hysteria: Yes/No
6. Any other:

D. Details of treatment

1. Surgical procedure:
2. Anesthesia
3. *Grid escharectomy:
4. *Escharectomy:
5. *Tangential excision and *STSG:
6. *STSG:
7. *Blood transfusion: Yes/No

E. General Observations

1. Complications:
   i. Hypovolumic shock
   ii. *Respiratory burn
   iii. Wound infection/septicemia:
   (a) Onset (b) Organisms

F. Dietary management

1. Feeding mode: Oral/oral and tube/tube only
2. Diet: experimental/hospital/any other
3. Type of nutrition support: total/supplemental
4. Feeding duration:

	Total	Supplemental	Quantity	Energy (kcal day^{-1})	Protein (g day^{-1})
Dates					

5. Occurrence of refeeding syndrome: Yes/No

G. Observations during the feeding periods

1. Weight (Wt.) of the patients (kg) and BMI (kg m^{-2}):

Days:	0	7	14	21	28	35	42	49	56	63	70	At discharge
Wt.:												
BMI:												

2. Triceps Skin fold thickness (mm):

Days:	0	7	14	21	28	35	42	49	56	63	70	At discharge

3. Mid-arm circumference (cm):

Days:	0	7	14	21	28	35	42	49	56	63	70	At discharge

4. Mid-arm muscle circumference (cm):

Days:	0	7	14	21	28	35	42	49	56	63	70	At discharge

5. Motion:
   i. Frequency/day:
   ii. Nature of the stool: watery/semisolid/explosive/frothy/normal
   iii. Color: yellowish/blackish/coffee ground/greenish/other
   iv. Quantity: small/moderate/large
   v. Constipation: Yes/No
6. Vomiting: yes/no. If yes:
   i. Frequency: ii. Nature: iii. Relation to food:
7. Nausea: Yes/No. If Yes, mild/moderate/severe
8. Abdominal pain: present/absent. If present, mild/moderate/severe
9. Distension of abdomen: present/absent
10. Fluid intake and urine output/day:
    i. Fluid intake _____mL ii. Urine output _____mL
11. Creatinine excretion in urine (24 h): mg day $^{-1}$
12. Ultrasound:**
13. Liver biopsy:**
14. Vital signs (regular examination):
    i. Pulse rate: ii. Respiratory rate:
    iii. Temperature: iv. BP
15. Systemic Examination:
    i. Cardiovascular system ii. Central nervous system
    iii. Alimentary system iv. Excretory system

16. Blood analyses:

Days:	0	7	14	21	28	35	42	49	56	63	70	At discharge
Hb												
WBC												
RBC												
Lymphocytes												
Monocytes												
Basophils												
Eisnophil												
Na												
K												
HCO_2												
Cl												
Total protein												
Albumin												
Prealbumin												
Globulin												
A/G												
BUN												
Creatinine												
Glucose: fasting and postpandrial												
Total cholesterol												
HDL-cholesterol												
LDL cholesterol												
TIBC												
PT												
Liver function test												
Wilson's diseases test**												
Viral marker**												
S. Ferritin												
Autoimmune disease test												
Any other												

*Information collected on burn patients only

**Information collected on cirrhotic patients with or without encephalopathy

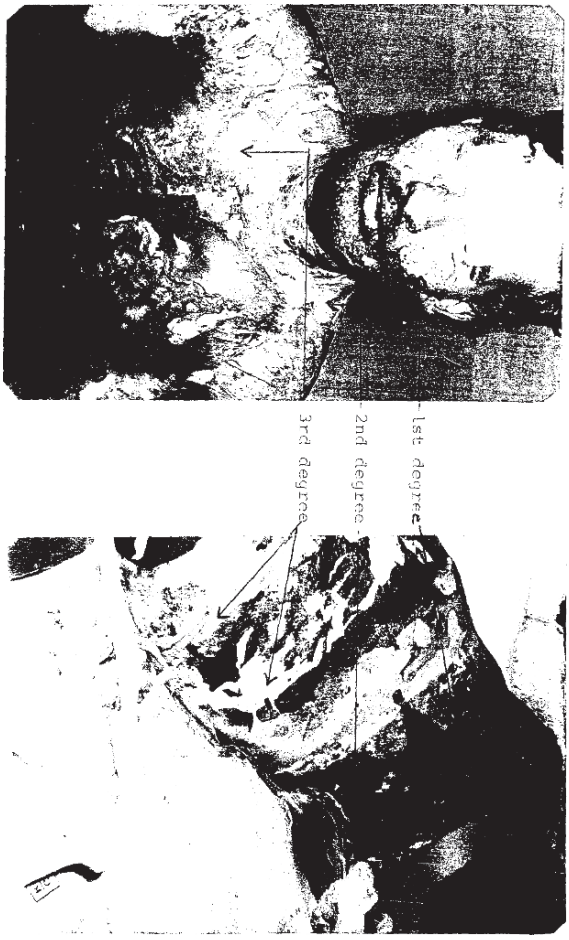
17. Immune Status: * i. Humoral immunity (IgG, IgA, IgM)
    ii. Cell-mediated immunity
18. Success of graft take: *
    i. Clinical judgment of 1 % of STSG at the end of 1 week.
19. Septic complication: SBP/pneumonia/UTI/sepsis/other
20. Details of diuretics: ** Week 1 Week 2 Week 3
21. Response of ascites to diuretics: ** (Wt./abd. girth)
22. Assessment of grade of hepatic encephalopathy: ** Grade: 1 2 3 4 (as per modified Parson-Smith scale of encephalopathy)
23. Progression of hepatic encephalopathy
    i. Week 1 ii. Week 2 iii. Week 3
24. General state of health: i. On admission. ii. At discharge
25: Duration of exclusive tube feeding _____days
26. Duration of hospital stay _____days
27. Duration of home enteral nutrition support
28. Morbidity: Yes/No
29. Mortality: Yes/No

H. General remarks on the enteral foods

1. By the patients:
2. By the guardian:
3. By the healthcare professionals:
   i. Physicians ii. Dietitians iii. Nurses

Appendix C

Photographs Showing Varying Degree of TBSA Burns



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Index