LECTURE COMPENDIUM
OF
NATIONAL TRAINING PROGRAMME
ON
Innovative Trends in Dairy and Food Products Formulation

OCTOBER 10 – 30, 2012

CENTRE OF ADVANCED FACULTY TRAINING
(Dairy Processing)

DAIRY TECHNOLOGY DIVISION
NATIONAL DAIRY RESEARCH INSTITUTE
(DEEMED UNIVERSITY)
KARNAL – 132 001, (HARYANA) INDIA
**Course Advisor**

Dr. A. A. Patel  
Director, Centre of Advanced Faculty Training in Dairy Processing and  
Head, Dairy Technology Division

**Course Director**

Dr. (Mrs.) Latha Sabikhi  
Senior Scientist, Dairy Technology Division

**Course Coordinators**

Mr. Sathish Kumar M.H.  
Scientist, Dairy Technology Division

Mr. Yogesh Khetra  
Scientist, Dairy Technology Division

NATIONAL DAIRY RESEARCH INSTITUTE  
(DEEMED UNIVERSITY)  
KARNAL – 132 001, (HARYANA)
Editing and Compilation

Dr. P. Narendra Raju
Dr. Latha Sabikhi
Dr. Kaushik Khamrui

Cover Page Designed By

Mr. Alok Chatterjee
Mr. Yogesh Khetra
Mr. Sathish Kumar, M.H.

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Indian food industry can be a global competitor in the food market, only if it keeps pace with the recent trends in food processing. The predominant factors that would enforce new trends would be continued productivity growth both at farm and processor level, competitiveness and economic survival, consumer needs and conveniences, socio-economic changes, enhanced buying power and continued evolution of market tastes.

The needs of market will determine the technology that will be required in the future. Health and functional foods, composite foods, special dietary foods, new ideas on by-product utilization, alternative processing techniques and the application of new ingredients as food processing aids provide clues on some emerging trends. Changed lifestyle will drive requirements for new products and for the improvement of current products. Increased desire for flavor, texture, variety, health, clean environment, product safety and economy will require significant changes in the way products are produced and presented. These changes will also place demands upon manufacturers and will enforce technological response.

Currently, the Indian Food Industry is at crossroads, as on the one hand we have enormous wealth in terms of surplus raw material and technical know-how and on the other hand we fall behind in terms of quality. It is imperative, therefore, that any new trends should be introduced after stringent quality measures are followed in all areas of production and post-harvest handling of food products to maintain strategic advantage in the world market. The Indian food industry will have to step forward with a positive and definite attitude towards implementing appropriate food safety measures to achieve this.

The time is ripe, therefore, to recapitulate our knowledge on the new trends that are being adopted in the food industry. I am certain that the National Training on Innovative Trends in Dairy and Food Product Formulation to be held under the aegis of the Centre of Advanced Faculty Training in Dairy Processing is aimed at achieving this. I hope this course will help to inculcate adequate motivation and an unquestionable resolve among the participants to herald in new trends in food product development, processing activities, packaging, quality maintenance and safety assurance.

(A.K. Srivastava)
Director, NDRI
ACKNOWLEDGEMENT

The Indian Council of Agricultural Research hailed the Division of Dairy Technology of NDRI as a Centre of Excellence in Dairy Technology for its Centre of Advanced Studies programme in the VIII Plan and subsequently renewed it in the X Plan based on admirable performance. The Centre has recently been rechristened as ‘Centre of Advanced Faculty Training (CAFT) in Dairy Processing’ by the ICAR. The CAFT-DP has been striving to improve and upgrade the teaching, research and training capabilities of the Division as well as to develop competence of the faculty members of the State Agriculture Universities by disseminating recent advances in the area of Dairy Processing. In the past, 25 training programmes in different areas of dairy processing have been successfully organized by CAFT. This is the 26th course on ‘Innovative Trends in Dairy and Food Products Formulation’ being conducted during October 10-30, 2012. As for the past course, this course also will be highly useful for the participating researchers and academicians in further developing their concepts in the area food formulation.

We express our gratitude to the Indian Council of Agricultural Research for placing the responsibility of CAFT (Dairy Processing) upon Dairy Technology Division. We also take this opportunity to thank Dr. Kusumakar Sharma, Asst. Director General (HRD) for his keen interest in this programme and timely release of funds.

We express our sincere thanks to Dr. A. K. Srivastava, Director, NDRI, Karnal for his constant encouragement and guidance and also for providing all necessary facilities for organizing this course. The continuing interest of Dr. Rishendra Verma, Joint Director (Research) and Dr. V.P. Singh, Joint Director (Academics), NDRI, Karnal, in the CAFT programme of the Division is gratefully acknowledged.

Dr. Latha Sabikhi, Senior Scientist and Course Director deserves a special mention for her diligent efforts that made the initiation of this programme a success. She was very ably supported by Mr. Sathish Kumar, M.H. and Mr. Yogesh Khetra, (Scientists, Course Co-ordinators) in this endeavour. Compilation of various lectures into a compendium, its editing and formatting is a very difficult task, especially when semester is in progress and teaching load is at its peak. We thank Dr. P.N. Raju and Dr. Kaushik Khamrui for their valuable assistance in formatting the manuscripts of the lectures for the compendium. I also thank Mr. Alok Chatterjee (Research Scholar) of the Division for providing valuable inputs in designing the cover page of the compendium.

We are highly indebted to the guest speaker, Mr. Naresh L., Manager – Product Development, Mother Dairy Fruit & Vegetable Private Limited, who contributed the lecture material well in time and traveled to Karnal to share his valuable expertise with the participants. We also thank M/s Nestle India for permitting the participants to visit their processing facilities at Samalkha.

We must convey our special thanks to the faculty of Dairy Technology, Dairy Chemistry, Dairy Microbiology, Dairy Engineering, Dairy Economics, Statistics & Management and English for submission of lectures and for actively participating in conducting the theory and practical classes. We specially thank the Chairmen and members of the different committees for their assistance and logistic support. We are grateful to the technical, ministerial and supporting staff of Dairy Technology Division for their contribution in day-to-day affairs of the CAFT programme.

Date: October 08, 2012. 

(A. A. Patel)
CENTRE OF ADVANCED FACULTY TRAINING IN DAIRY PROCESSING

COMMITTEES FOR ORGANIZATION OF THE
National Training
ON
INNOVATIVE TRENDS IN DAIRY AND FOOD PRODUCTS FORMULATION
From 10th to 30th October, 2012

ORGANISING COMMITTEE
Dr. A.A. Patel (Director, CAFT)
Dr. Latha Sabikhi (Course Director)
Mr. Yogesh Khetra (Course Co-ordinator)
Mr. Sathish Kumar, M.H. (Course Co-ordinator)

RECEPTION COMMITTEE
Dr. S.K. Kanawjia (Chairman)
Mr. Yogesh Khetra

TECHNICAL COMMITTEE
Dr. V.K. Gupta (Chairman)
Dr. Latha Sabikhi
Dr. Kaushik Khamrui
Dr. P.N. Raju

HOSPITALITY COMMITTEE
Dr. R.R.B. Singh (Chairman)
Dr. Prateek Sharma
Mr. G.S. Meena

TOUR COMMITTEE
Dr. A.K. Singh (Chairman)
Mr. H.C. Devaraja

PURCHASE COMMITTEE
Dr. D.K. Thompkinson (Chairman)
Mr. Sathish Kumar M.H.
## Programme

### 10.10.2012 (Wednesday)

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<th>Time</th>
<th>Activity</th>
<th>Presenter/ Organizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:00 AM-10:30 AM</td>
<td>Registration</td>
<td>Mr. Y. Khetra</td>
</tr>
<tr>
<td>10:30 AM-12:00 Noon</td>
<td>Inauguration</td>
<td></td>
</tr>
<tr>
<td>12:05 PM-01:00 PM</td>
<td>Introduction of CAFT (DP) to course participants</td>
<td>Dr. (Mrs.) Latha Sabikhi</td>
</tr>
<tr>
<td>01:00 PM-02:00 PM</td>
<td>LUNCH</td>
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</tr>
<tr>
<td>02:00 PM-03:00 PM</td>
<td>New product development in dairy and food industry-trends and scenario</td>
<td>Dr. A.A. Patel</td>
</tr>
<tr>
<td>03:00 PM-05:00 PM</td>
<td>Visit to model dairy plant, cattle yard and ATIC</td>
<td>Mr. Ram Swaroop / Mr. S.K. Kharb</td>
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</tbody>
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### 11.10.2012 (Thursday)

<table>
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<tr>
<th>Time</th>
<th>Activity</th>
<th>Presenter/ Organizer</th>
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<tbody>
<tr>
<td>09:30 AM-10:30 AM</td>
<td>Functional dairy foods-Prospects and constraints</td>
<td>Dr. A.K. Singh</td>
</tr>
<tr>
<td>10:30 AM-11:30 AM</td>
<td>Herbal bioactives and their delivery systems for health foods formulation</td>
<td>Mr. P. Sharma</td>
</tr>
<tr>
<td>11:30 AM-11:45 AM</td>
<td>TEA BREAK</td>
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<tr>
<td>11:45 AM-12:45 PM</td>
<td>Novelty in Milk Based Confectionary Products</td>
<td>Mr. Kutumb Rao</td>
</tr>
<tr>
<td>01:00 PM-02:00 PM</td>
<td>LUNCH</td>
<td></td>
</tr>
<tr>
<td>02:00 PM-05:00 PM</td>
<td>Preparation of Nanoemulsions with bioactives—Practical</td>
<td>Mr. P. Sharma</td>
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</table>

### 12.10.2012 (Friday)

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>09:30 AM-10:30 AM</td>
<td>Mode of action of probiotics in humans</td>
<td>Rashmi, H.M.</td>
</tr>
<tr>
<td>10:30 AM-11:30 AM</td>
<td>Microencapsulation: Strategy to increase viability of probiotics</td>
<td>Dr. (Mrs.) Latha Sabikhi</td>
</tr>
<tr>
<td>11:30 AM-11:45 AM</td>
<td>TEA BREAK</td>
<td></td>
</tr>
<tr>
<td>11:45 AM-12:45 PM</td>
<td>Trends and Innovations in Ice Cream Formulation</td>
<td>Mr. Naresh, Mother Dairy</td>
</tr>
<tr>
<td>01:00 PM-02:00 PM</td>
<td>LUNCH</td>
<td></td>
</tr>
<tr>
<td>02:00 PM-05:00 PM</td>
<td>Formulation of synbiotic Ice Cream—Practical</td>
<td>Dr. R.R.B. Singh/ Ms. Vidhu Yadav</td>
</tr>
</tbody>
</table>

### 13.10.2012 (Saturday)

Visit to Amritpur Kalan village for interaction with women Self Help Group

### 14.10.2012 (Sunday) Holiday

### 15.10.2012 (Monday)

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Presenter/ Organizer</th>
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<tbody>
<tr>
<td>09:30 AM-10:30 AM</td>
<td>Novelty in Traditional Dairy Products</td>
<td>Dr. K. Khamrui</td>
</tr>
<tr>
<td>10:30 AM-11:30 AM</td>
<td>Advances in infant food formulations</td>
<td>Dr. D.K. Thompkinson</td>
</tr>
<tr>
<td>Time</td>
<td>Event</td>
<td>Speaker</td>
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<tr>
<td>11:30 AM-11:45 AM</td>
<td>TEA BREAK</td>
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<tr>
<td>11:45 AM-12:45 PM</td>
<td>Designer Dairy Foods</td>
<td>Dr. (Mrs.) Latha Sabikhi</td>
</tr>
<tr>
<td>01:00 PM- 02:00 PM</td>
<td>LUNCH</td>
<td></td>
</tr>
<tr>
<td>02.00 PM- 05.00 PM</td>
<td>Statistical analysis for product development – Theory and Practical</td>
<td>Dr. R. Malhotra</td>
</tr>
<tr>
<td>09:30 AM-10:30 AM</td>
<td>Fortification of milk with minerals and vitamins</td>
<td>Dr. Sumit Arora</td>
</tr>
<tr>
<td>10:30 AM-11:30 AM</td>
<td>Composite Foods: Technology and Related Issues</td>
<td>Dr. A.K. Singh</td>
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<tr>
<td>11:30 AM-11:45 AM</td>
<td>TEA BREAK</td>
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<tr>
<td>11:45 AM-12:45 PM</td>
<td>Protein rich foods</td>
<td>Dr. Rajesh Bajaj</td>
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<td>01:00 PM-02:00 PM</td>
<td>LUNCH</td>
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<tr>
<td>02:00 PM-05:00 PM</td>
<td>Fortification of milk with micronutrients -- Practical</td>
<td>Dr. Sumit Arora</td>
</tr>
<tr>
<td>09:30 AM-10:30 AM</td>
<td>Way forward with intense sweeteners for Diabetic food formulations</td>
<td>Dr. P.N. Raju</td>
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<tr>
<td>10:30 AM-11:30 AM</td>
<td>Recent applications of scrapped surface heat exchangers in dairy foods manufacture</td>
<td>Dr. A.K. Dodeja</td>
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<tr>
<td>11:30 AM-11:45 AM</td>
<td>TEA BREAK</td>
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<tr>
<td>11:45 AM-12:45 PM</td>
<td>Advances in membrane processing for production of novel ingredients</td>
<td>Dr. V.K. Gupta</td>
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<tr>
<td>01:00 PM-02:00 PM</td>
<td>LUNCH</td>
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<tr>
<td>02:00 PM-05:00 PM</td>
<td>Preparation of low calorie <em>misti dahi</em> - Practical</td>
<td>Dr. P.N. Raju</td>
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<tr>
<td>09:30 AM-10:30 AM</td>
<td>Product evaluation and test marketing</td>
<td>Dr. Raka Saxena</td>
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<tr>
<td>10:30 AM-11:30 AM</td>
<td>Marketing research techniques: Communicating customer voice to product development team</td>
<td>Dr. Smita Sirohi</td>
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<tr>
<td>11:30 AM-11:45 AM</td>
<td>TEA BREAK</td>
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<tr>
<td>11:45 AM-12:45 PM</td>
<td>Patenting and Technology transfer: Opportunities and challenges</td>
<td>Dr. Y.S. Rajput</td>
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<tr>
<td>01:00 PM-02:00 PM</td>
<td>LUNCH</td>
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<tr>
<td>02:00 PM-05:00 PM</td>
<td>Preparation of fruit and milk smoothie – Practical</td>
<td>Mr. Sathish Kumar, M.H</td>
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<tr>
<td>09:30 AM-10:30 AM</td>
<td>Milk derived bioactive peptides – potential ingredients for food formulations</td>
<td>Dr. Bimlesh Mann</td>
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<tr>
<td>10:30 AM-11:30 AM</td>
<td>Basics of scientific writing and communication</td>
<td>Dr. Meena Malik</td>
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<tr>
<td>11:30 AM-11:45 AM</td>
<td>TEA BREAK</td>
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<tr>
<td>11:45 AM-12:45 PM</td>
<td>Recent trends in biofunctional dairy beverages</td>
<td>Dr. Shilpa Vij</td>
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<tr>
<td>01:00 PM-02:00 PM</td>
<td>LUNCH</td>
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<tr>
<td>02:00 PM-05:00 PM</td>
<td>Preparation of protein enriched milk through membrane technology</td>
<td>Dr. V.K. Gupta / Mr. G.S. Meena</td>
</tr>
<tr>
<td>09:30 AM-10:30 AM</td>
<td>Validation of health claims through animal bioassay – Theory</td>
<td>Dr. Suman Kapila</td>
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<tr>
<td>10:30 AM-10:45 AM</td>
<td>TEA BREAK</td>
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<tr>
<td>10:45 AM-01:00 PM</td>
<td>Validation of health claims through animal bioassay – Practical</td>
<td>Dr. Suman Kapila</td>
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<td>01:00 PM-02:00 PM</td>
<td>LUNCH</td>
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<tr>
<td>02:00 PM-05:00 PM</td>
<td>Preparation of composite dairy foods – Practical</td>
<td>Dr. A.K. Singh</td>
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</table>
### 23.10.2012 (Tuesday)

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:30 AM</td>
<td>Extrusion: Novel technology for snack foods</td>
<td>Dr. R.R.B. Singh</td>
</tr>
<tr>
<td>10:30 AM</td>
<td>Research and developments in space foods</td>
<td>Mr. Devaraja, H.C.</td>
</tr>
<tr>
<td>11:30 AM</td>
<td>TEA BREAK</td>
<td></td>
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<tr>
<td>11:45 AM</td>
<td>Cost cutting measures in new product development</td>
<td>Dr. Latha Sabikhi</td>
</tr>
<tr>
<td>01:00 PM</td>
<td>LUNCH</td>
<td></td>
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<tr>
<td>02:00 PM</td>
<td>Preparation of protein rich extruded snacks -- Practical</td>
<td>Dr. A.K. Singh</td>
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### 24.10.2012 (Wednesday) Dusshera/Navarathri celebration

### 25.10.2012 (Thursday)

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>09:30 AM</td>
<td>Overview of food safety standards – Global perspective</td>
<td>Dr. Naresh Goel</td>
</tr>
<tr>
<td>10:30 AM</td>
<td>Personnel hygiene and safety measures to meet occupational standards</td>
<td>Dr. Vaishali Goel</td>
</tr>
<tr>
<td>11:30 AM</td>
<td>TEA BREAK</td>
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<tr>
<td>11:45 AM</td>
<td>Emerging food-borne pathogens - case studies</td>
<td>Dr. Chand Ram</td>
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<tr>
<td>01:00 PM</td>
<td>LUNCH</td>
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<tr>
<td>02:00 PM</td>
<td>Biosensor based rapid techniques for identification of food pathogens – Theory and Practical</td>
<td>Dr. Naresh Goel / Mr. Raghu, H.V.</td>
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### 26.10.2012 (Friday)

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>09:30 AM</td>
<td>Advances in fermented milk products technology</td>
<td>Dr. S.K. Kanawjia</td>
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<tr>
<td>10:30 AM</td>
<td>Protein hydrolysates for better texture of dairy products</td>
<td>Dr. Bimlesh Mann</td>
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<tr>
<td>11:30 AM</td>
<td>TEA BREAK</td>
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<tr>
<td>11:45 AM</td>
<td>Innovations in educational system for improved HRD in the area of food science technology and nutrition</td>
<td>Dr. Alok Jha</td>
</tr>
<tr>
<td>01:00 PM</td>
<td>LUNCH</td>
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<tr>
<td>02:00 PM</td>
<td>Determination and quantification of bioactive peptides -- Practical</td>
<td>Dr. Bimlesh Mann</td>
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### 27.10.2012 (Saturday)

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>09:30 AM</td>
<td>Innovations in Ready-to-serve beverages</td>
<td>Mr. Sathish Kumar, M.H.</td>
</tr>
<tr>
<td>10:30 AM</td>
<td>Formulations of cereal based fermented beverages</td>
<td>Mr. Y. Khetra</td>
</tr>
<tr>
<td>11:30 AM</td>
<td>TEA BREAK</td>
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<tr>
<td>11:45 AM</td>
<td>Enzymatic hydrolysis of milk protein</td>
<td>Mr. G.S. Meena</td>
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<td>01:00 PM</td>
<td>LUNCH</td>
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<tr>
<td>02:00 PM</td>
<td>Microencapsulation -- Practical</td>
<td>Mr. Sathish Kumar, M.H.</td>
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### 28.10.2012 (Sunday) Holiday

### 29.10.2012 (Monday)

<table>
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<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>09:30 AM</td>
<td>Applications of bacteriocins in dairy food formulation</td>
<td>Dr. R.K. Malik</td>
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<tr>
<td>10:30 AM</td>
<td>Formulation of health foods to combat chronic and degenerative diseases</td>
<td>Dr. K. Khamrui</td>
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<tr>
<td>11:30 AM</td>
<td>TEA BREAK</td>
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<tr>
<td>11:45 AM</td>
<td>Omega-3, CLA and phytosterols for improving functionality of dairy products</td>
<td>Dr. Vivek Sharma</td>
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<tr>
<td>01:00 PM</td>
<td>LUNCH</td>
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<tr>
<td>02:00 PM</td>
<td>Formulation of Functional Cheese Spreads—Theory &amp; Practical</td>
<td>Dr. S.K. Kanawjia/Mr. Apurba Giri</td>
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### 30.10.2012 (Tuesday)

<table>
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<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>09:30 AM</td>
<td>Novel applications of starter cultures</td>
<td>Dr. S.K. Tomar</td>
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<tr>
<td>10:30 AM</td>
<td>Packaging – Tools to improve sales and profits</td>
<td>Dr. P.N. Raju</td>
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<tr>
<td>11:30 AM</td>
<td>TEA BREAK</td>
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<tr>
<td>01:00 PM</td>
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<tr>
<td>02:00 PM</td>
<td>Valedictory session</td>
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Trends in New Product Development with Reference to Functional Foods

A.A. Patel and A.K. Singh
Dairy Technology Division

1. Introduction

Product development is crucial to the growth of dairy and food industry. There is no denying the fact that certain products have been being marketed almost unchanged since their introduction several decades ago. Examples in the dairy category of foods are pasteurized milk, table butter, several varieties of cheese, milk powder, etc. However, there have been several ‘new products’ such as low-fat spreads, dairy desserts, UHT-treated flavoured milk, ice cream novelties, etc. brought onto the market in the past. The most common driving force behind new product development is expanding the product portfolio of the organization aimed at business growth. There may several other reasons food manufacturers may take to new products. Notable among these can be considered to be convenience products such as fat spreads and extended shelf-life products such as thermized yoghurt and shrikhand. Changing consumer demands -- perceived, potential or real, is perhaps the most significant factor leading to new product development. ‘Health foods’ or ‘functional foods’ represent a very valuable group of products in this context.

Rapidly changing life style and the rise of incidence of life-style related disorders viz., diabetes, obesity, coronary heart disease, etc. have been the principal causes of development of new food products that can address the needs of the concerned consumer groups. Physiologically functional foods are fast growing in demand with more and more consumers becoming aware about the relationship between food and well being. Thus, the increasing awareness among consumers to know which specific molecules present in their food possess disease preventive or curative properties has led to the concept of functional foods which represent, in terms of their contents, much more than the conventional products’ compositional characteristics. Now the attention of scientific investigations has moved towards exploring the role of biologically active components on human health. Basic temptation in human being towards nature and the products that are natural, for every little disturbances related to health resulted in flourishing of market with products containing various therapeutic ingredients. Functional foods, pharma foods, designer foods and nutraceuticals are synonymous for foods that can prevent and treat diseases. Epidemiological studies and randomized clinical trials carried out in different parts of the world have demonstrated or at least suggested numerous health effects related to functional food consumption, such as reduction of cancer risk, improvement of heart health, enhancement of immune functions, lowering of menopause symptoms, improvement of gastrointestinal health, anti-inflammatory effects, reduction of blood pressure, antibacterial & antiviral activities, reduction of osteoporosis etc.

Nutritional significance of milk molecules is well documented and increasing cases of cancers, coronary heart diseases, osteoporosis and many other chronic diseases, have been attributed to our diet. But beyond these known nutrients i.e. vitamins, proteins, milk and milk constituents have clearly more to offer and scientists are scurrying to discover exactly which milk components might fend off specific diseases. An ever-expanding array of previously unknown such molecules with hard to pronounce names is being uncovered. But there exact metabolic role and how these can be utilized in designer food, need to be elucidated.
All over the world there has been growing demand for functional foods. Currently Japan leads the world in the functional-food production, with production and consumption of more than 100 such products.

2. The definition of ‘Functional Food’

The term “functional food” was first used in Japan, in the 1980s, for food products fortified with special constituents that possess advantageous physiological effects. Functional foods may improve the general conditions of the body or decrease the risk of some diseases and could even be used for curing some illnesses. Although the term “functional food” has already been defined by many scientific and regulatory bodies, so far there is no universally accepted definition for this group of food. In most countries there is no legislative definition of the term and drawing a borderline between conventional and functional foods is challenging even for nutrition and food experts. To date, a number of national authorities, academic bodies and the industry have proposed definitions for functional foods. And the general consensus seems to be emerging towards ‘Functional foods’ defined as “foods that, by virtue of the presence of physiologically active components, provide a health benefit beyond basic nutrition”.

The Institute of Medicine's Food and Nutrition Board (IOM/FNB, 1994), defined functional food as “any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains”. To qualify as a functional food, it should meet the following three conditions:

- It is a food (not capsule, tablet or powder) derived from naturally occurring ingredients.
- It can and should be consumed as part of daily diet.
- When ingested, it should serve to regulate a particular body process, such as
  - Improvement of biological defense mechanisms
  - Prevention and recovery of specific disease.
  - Control of mental and physical conditions
  - Retarding the ageing process.

Some examples of functional foods are given in Table 1.

3. Significance of functional foods

In recent years, there has been a vast and rapidly growing body of scientific data showing that diet plays an important part in diseases. Diet is thought to contribute to six of the 10 leading causes of death. Nutrients and nonnutritive food components have been associated with the prevention and/or treatment of chronic diseases such as cancer, coronary heart disease (CHD), diabetes, hypertension, and osteoporosis. According to an estimate about 70% of certain cancers are directly related to the type of food we eat. As the data supporting the role of diet in health promotion and disease prevention continue to mount, it is likely that the quantity of enhanced foods will expand substantially. There is an increasing demand by consumers for quality of life, which is fueling the nutraceutical revolution. Functional foods are viewed as one option available for seeking cost-effective health care and improved health status. Moreover, the large segment of the population is aging and considerable health care budget in most countries is focused on treatment rather than prevention. Thus, the use of nutraceuticals in daily diets can be seen as means to
reduce escalating health care costs that will contribute not only to a longer lifespan, but also more importantly, to a longer health span.

Table 1. Major categories of functional foods

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
<th>Example</th>
</tr>
</thead>
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<tr>
<td>Fortified product</td>
<td>A food fortified with additional nutrients</td>
<td>Milk powder fortified with vitamin D</td>
</tr>
<tr>
<td>Enriched products</td>
<td>A food with added new nutrients or components not normally found in a particular food</td>
<td>Margarine with plant sterol ester, probiotics, prebiotics</td>
</tr>
<tr>
<td>Altered products</td>
<td>A food from which a deleterious component has been removed, reduced or replaced with another substance with beneficial effects</td>
<td>Lactose hydrolyzed milk</td>
</tr>
<tr>
<td>Enhanced commodities</td>
<td>A food in which one of the component has been naturally enhanced through special growing condition, new feed composition, genetic manipulation, or otherwise</td>
<td>CLA enhancement in milk through feeding of green fodders</td>
</tr>
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</table>

4. Trends in functional foods and nutraceuticals

Companies involved in investigating and developing nutraceutical products mainly belong to the food (55%) and pharmaceutical (35%) industries. Strategic alliances are already in place among pharmaceutical and food companies. In India, nutraceuticals are marketed as Indian System of Medicine (ISM) drugs under the OTC category. No clinical validation of their safety or efficacy is required if therapeutically usefulness is mentioned in the text. Currently, functional foods market is estimated at $70 billion or 4% of processed food market and is growing at three times the rate. In developed markets, higher consumer awareness on health and wellness is being addressed through product innovations and marketing prowess of large players. While the ageing population needs more engineered foods the younger population is demanding more fortified foods to get extra energy. Health-related issues -obesity and coronary heart disease -are forcing food processors to launch campaigns to promote low carbohydrate diets or other such foods. Japan is the single largest market with per capita consumption of $140+ with the US and Europe following at $95 and $60 respectively. Despite static or reducing population, functional foods will continue to retain the market (or grow) due to growing health concerns. Regulations have ensured the segment's organized growth.

There are a lot of products sold in the name of nutraceuticals in the Indian market. Close to around 100 products are even listed on the Internet along with the global companies and around 20 Indian companies have a record of producing nutraceuticals and marketing them globally. India is relatively a new market. The size of the Indian nutraceutical market is estimated to be about Rs 1,600 crore in 2001. All major pharma players are in the process of entering this market. The level of exports from India is still small, estimated to be perhaps less than Rs 750 crore, if one excludes Psyllium. The major markets for India are the US, Europe and Japan. India can become leader in this field as we hold key expertise as well as we are rich with the biodiversity.
5. Milk based functional foods and nutraceuticals

Since time immemorial, dairy products have been an integral part of human diet. Milk is the only food, which has got the power to sustain life in all the stages of development, and is considered an important part of a balanced diet. Besides being a source of quality proteins and energy–rich fat, it contains important micronutrients like calcium, potassium, sodium, magnesium and vitamins, which are vital for overall development of the human body. Also, several health attributes are associated with milk or its constituents, such as the role of calcium in controlling hypertension and colonic anticarcinogenicity, protective roles of carotenoids and conjugated Linoleic acid (CLA) against cancers (Table 2). Butyric acid, the short chain fatty acid of milk fat has been shown to regulate cell growth and enhance the anti-tumor activities. Certain minor milk components either naturally occur or formed during processing have also been endowed with many unique health benefits. Examples include lactoferrin, lactulose, galacto-oligosaccharides (GOS), β-lactoglobulin, and bioactive peptides. Some of the important segments of functional dairy foods and nutraceuticals have been discussed hereunder.

Table 2. Examples of functional components in milk and milk products

<table>
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<tr>
<th>Class/Components</th>
<th>Source</th>
<th>Potential Benefit</th>
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<tbody>
<tr>
<td>Probiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacilli,</td>
<td>yogurt, other dairy and non-dairy applications</td>
<td>may improve gastrointestinal health and systemic immunity</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty Acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conjugated Linoleic Acid (CLA)</td>
<td>Fat rich dairy products, fermented milk products</td>
<td>Anti-cancer, Anti-atherosclerosis</td>
</tr>
<tr>
<td>Whey Proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-Lactoglobulin</td>
<td>Whey</td>
<td>Enhance glutathione synthesis</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Whey, Colostrums</td>
<td>Anti-bacterial, increase bioavailability of iron</td>
</tr>
<tr>
<td>Prebiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lactulose</td>
<td>Heated milks, Synthesized from lactose</td>
<td>Bifodgenic factor, improve GIT conditions in infants, laxative, prevent allergy</td>
</tr>
<tr>
<td>Galacto-oligosaccharides</td>
<td>Fermented foods, activity of microbes</td>
<td>Promote growth of probiotic bacteria, anticancer, increase mineral bioavailability</td>
</tr>
<tr>
<td>Bioactive Peptides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caseinophosphopeptides</td>
<td>Fermented milks, Proteolysis of casein</td>
<td>Mineral binding specially calcium</td>
</tr>
<tr>
<td>Csomorphins</td>
<td>Proteolysis of α &amp; β-casein</td>
<td>Increase intestinal water &amp; electrolyte absorption, increase GI transit time</td>
</tr>
</tbody>
</table>

5.1 Probiotic dairy foods

Human gastrointestinal tract (GIT) harbour more than 100 trillion microorganisms belong to 400 different bacterial species. The number cells are almost 10 times than the rest of the body cells. A delicate balance exists between beneficial and harmful bacteria [resent in GIT and any disturbance may lead to abnormalities. About 70% of the body’s immune system is localized in GIT. Incorporation of beneficial bacteria into foods to counteract
harmful organisms in the GIT has been the most visible component of this new area. Such microorganisms are termed as “Probiotics”. There is growing scientific evidence to support the concept that beneficial gut microflora may provide protection against gastrointestinal disorders including gastrointestinal infections, inflammatory bowel diseases, and even cancer.

The basis for selection of probiotic micro-organisms include safety, functional aspects (survival, adherence, colonization, antimicrobial production, immune stimulation, antigenotoxic activity and prevention of pathogens) and technological details such as growth in milk and other food base, sensory properties, stability, phage resistance and viability. Newer avenues as carriers of probiotic organisms are being sought. Fermented milk pridcuts being a 'live' food, is potentially an excellent vehicle for these beneficial microbial cultures. Recognizing the beneficial properties of probiotic organisms and challenged with the possibility that these organisms may produce end products that may be different from those produced by the normal starters in these products; several attempts have been made to manufacture probiotic milk products like probiotic dahi, probiotic cheese, probiotic yoghurt and yoghurt drinks. Probiotic dahi developed at our Institute containing Lactobacillus acidophilus and Lactobacillus casei was found to delay the onset of glucose intolerance, hyperglycemia, dyslipidemia, and oxidative stress in high fructose induced diabetic rats. It indicates reduced risk of diabetes and its complications.

5.2 Fortified milk products

Milk in its natural form is almost unique as a balanced source of man’s dietary need. The various steps in processing and storage have a measurable impact on some specific nutrients. Milk also provides a convenient and useful vehicle for addition of certain nutrients to our diet and has following benefits:

- Easier quality control measure implementation
- Wider consumption by all age groups
- Cost is affordable by target population.
- Higher stability and bioavailability of the added micronutrients
- Addition of fortificants usually caused minimum change in colour, taste and appearance.

Liquid milk fortification with vitamins A and/D is mandatory in several countries. β-carotene is added as a colour-enhancing agent to some milk products such as butter. Dried milk is often fortified with vitamins A and D, calcium, and iron. Milk based infant formula and weaning foods are fortified with a range of vitamins, minerals, and other nutrients such as polyunsaturated fatty acids. Powdered milk used for complementary feeding in Chile is fortified with vitamin C, iron, copper and zinc. However, the milk fortification usually impaired its sensory and processing quality characteristics. Moreover, bio-availability of fortified nutrients is another major concern. Investigations carried out at NDRI suggest possibilities of fortification of liquid milk with calcium and iron.

5.3 Whey proteins and peptides

Whey proteins are termed as “wonder proteins” and may find applications in nutritional, functional, dietetic, sports and infant foods. They possess excellent amino acids profile, high PER and biological value, easier digestibility and assimilability, bland taste and
excellent functional properties. Dietary whey proteins have a number of putative, biological effects when ingested. The ability of whey proteins to increase the level of natural anti-oxidants within the body and possibly in stabilizing DNA during cell division is emerging as premier contribution to population health. The anticarcinogenic properties of whey proteins are related to compounds rich in sulphur containing amino acids, methionine and cysteine. They contain γ-glutamyl-cysteine residue, which makes cysteine readily available for synthesis of glutathion, (γ-glutamyl- cysteinyl- glycine) a strong xenobiotic deactivating and anti-neoplastic agent. Methionine is utilized for glutathion synthesis in times of cysteine deficiency and it also acts as methyl donor. Hypomethylation of a DNA is an important risk factor for cancer at number of sites. Glutathion is believed to act as an antioxidant, anticarcinogenic and in stabilization and repair of DNA.

6. Challenges in development of functional dairy foods

In India, we have traditional products touted as functional but have little scientific validation. Regulations will thus have to evolve to promote R&D, ensure validation and prevent exploitation of consumers. Companies will also have to be sincere and honest in their claims while marketing and communicating with consumers till appropriate regulations for scientific validation are evolved. Processors will need to provide an optimal merger between taste, convenience and health attributes. Companies will require expert knowledge in flavour masking fortification know-how and delivery systems.

6.1 Technological Challenges

Four different technological hurdles have to be overcome before a product containing bioactive substances is ready to consider marketing:

- Isolation of the desired components,
- Pre-establishment of the biological activity,
- Incorporation of the bioactive components into a formulated product,
- Verification of efficacy and safety of final product.

Such a sequence of experimental events is also required for the introduction of new food additives; additional is a thorough proof of the claimed benefit of the bioactive components. This applies especially when the bioactive component is a completely new substance never consumed before in significant amounts. Separation, purification and production at industrial level of such nutraceuticals must be thought in terms of integrated and high added value. Membrane technologies (MF, UF, NF) provide key opportunities to manufacture milk nutraceuticals in native state. The incorporation of bioactive components into processed foods, its delivery and bioavailability are other important issues that need reprisal.

6.2 Scientific validation of functional foods

The scientific evidence for functional foods and their physiologically active components can be categorized into four distinct areas: (a) clinical trials, (b) animal studies, (c) experimental in vitro laboratory studies, and (d) epidemiologic studies. Much of the current evidence for functional foods lacks well-designed clinical trials; however, the foundational evidence provided through the other types of scientific investigation is substantial for several of the functional foods and their health-promoting components.
6.3 Safety issues

Although increasing the availability of healthful foods including functional foods in the diet is critical to ensuring a healthier population, safety is a critical issue. The optimal levels of the majority of the biologically active components currently under investigation have yet to be determined. The benefits and risks to individuals and populations as a whole must be weighed carefully when considering the widespread use of physiologically-active functional foods. Knowledge of toxicity of functional food components is crucial to decrease the risk: benefit ratio. The safety issues related to probiotic microorganisms that has to be considered include

- intrinsic properties of the probiotic strains
- pharmacokinetics of probiotic strains
- interaction between probiotic strains and the host
- knowledge of toxicity of functional food components is crucial to decrease the risk: benefit ratio.

7. Conclusion

Consumer awareness about the relationship between diet and health has led to continuously growing demand for functional foods. Rapid advances in science and technology, increasing healthcare costs, changes in food laws affecting label and product claims, an aging population, and rising interest in attaining wellness through diet are among the factors fueling interest in functional foods. Credible scientific research indicates many potential health benefits from milk components. Development of functional foods needs take into account a number of issues ranging from material selection, process parameters, sensory acceptance as well as validation of the intended health virtues of the food. While considerable progress has been made in this regard, much more needs to be done in this area.
1. Introduction

Research interventions have established that novel value-added products can be derived from milk and milk products by altering milk composition for processing and/or animal and human health by employing nutritional and genetic approaches. Milk composition can be altered by nutritional management or through the manipulation of naturally occurring genetic variation among cattle. By combining these two approaches, researchers are now hoping to develop 'designer milk' tailored to consumer preferences or rich in specific milk components that have implications in health as well as processing. A greater proportion of unsaturated fatty acids in milk fat, reduced lactose content for lactose-intolerant people, and/or milk free from β-lactoglobulin (β-LG) would benefit human diet and health. Alteration of primary structure of casein to improve technological properties of milk, production of high protein milk, engineering milk meant for cheese manufacturing that leads to accelerated curd clotting time, increased yield and/or more protein recovery, milk containing nutraceuticals and replacement for infant formula would be helpful processing aids.

2. Carbohydrate modification

Lactose, the major milk sugar, regulates the osmotic process of lactation, thus causing the movement of water into milk. This carbohydrate is synthesized in the secretory vesicles of the mammary glands by the lactose synthase complex. As lactose cannot diffuse out of the vesicles, it draws water into the vesicles by osmosis. Thus, the volume of milk produced is directly dependent on the amount of lactose synthesized.

Lactose cannot be transported to the bloodstream directly and can be absorbed only after its enzymatic hydrolysis to the monosaccharides glucose and galactose by intestinal lactase (β-galactosidase). As milk is a major component in the human diet, lactose intolerance (caused by the absence of this enzyme) limits the use of a valuable nutritional source for many people. In addition, since milk can provide much of the calcium we require to maintain bone health, lactose intolerance can also be associated with osteopenia in later life.

Lactose intolerance can also be tackled through the use of β-galactosidase-replacement (preharvest) or hydrolyzed low-lactose (postharvest) products. Besides the nutritional advantage, a reduction in milk lactose content could also benefit the industry with less volume to transport, better milk coagulation, and less effluent production. The complete removal of lactose from milk creates milk that is extremely viscous, containing very little water. It is extremely difficult to extract this milk from the mammary gland, making the milking process difficult and painful for the animal.

The pre-harvest methodologies of reducing lactose either by the removal of α-lactalbumin (α-LA) and gene 'knock-out' methodologies or by introducing the lactase enzyme into milk via mammary gland specific expression have not been successful, as the former
resulted in highly viscous milk and the latter, in milk with high osmotic pressure due to the mono-saccharides produced. An in vivo technique for low-lactose milk production by generating transgenic mice that selectively produce a biologically active lactase in their milk was later demonstrated. In contrast to previous results, the lactose content reduced while retaining most of the monosaccharides of the milk.

3. Fat modification

Although it is not possible to achieve the 'ideal' milk fat composition (<10% polyunsaturated fatty acids, <8% saturated fatty acids, and >82% monosaturated fatty acids), manipulation of composition of milk fat is possible through feeding practices for dairy cows. Trials conducted at the University of Alberta (US) have demonstrated that adding a blend of canola oil and linseed oil to the cow's diet enhances the nutritional quality of milk fat and improves the spreadability of butter. A 'designer cow' called Daisy which can produce semi-skimmed (half-fat) milk (diet: dehusked oats) and soft-spreading butter that spreads straight from the refrigerator (diet: rapeseed oil) has been bred in Britain. Similarly, restricted quantities of fish oil, fish meal or plankton added to the cow's diet of grass or silage can produce milk rich in omega-3 fatty acids. Dietary fats such as corn oil fed to cows in the protected form results in the production of milk with substantially increased levels of conjugated linoleic acid (CLA), which reportedly suppresses carcinogens, inhibits proliferation of leukemia and colon, prostate, ovarian, and breast cancers.

In changing the fat composition, targeting enzymes that influence the synthesis of fat is important. As an example, reduction of acetyl CoA carboxylase that regulates the rate of fat synthesis within the mammary gland would translate to a drastic reduction in the fat content of milk and reduce the energy required by the animal to produce milk. Similarly, genetic variants of stearoyl-CoA desaturase has an influence on degree of unsaturation and on concentration of conjugated linolenic acid in the milk fat.

The type of fatty acids present in milk fat can influence the flavor and physical properties of dairy products. There are reports that butter produced from cows fed high oleic sunflower seeds and regular sunflower seeds were similar in flavour and texture to the control butter. Extruded soybean and sunflower diets yielded a Cheddar cheese that had higher concentrations of unsaturated fatty acids while maintaining flavor, manufacturing, and storage characteristics similar to that of control cheese. It is also beneficial from a safety point of view, as the accumulation of fatty acids, namely C12, C14, C18:1 and C18:2 enhanced the safety of cheeses against Listeria monocytogenes and Salmonella typhimurium.

4. Protein modification

Improved amino acid profile by the addition of L-taurine, L-leucine and L-phenylalanine offers additional nutritional benefits. Active whey peptides such as glyco-macro-peptide (GMP) is valuable in diet preparations for children with phenylketonuria (PKU) disorder, a condition that can lead to mental retardation if not treated early. Those with this rare metabolic disorder have an impaired ability to metabolise phenylalanine, a component of most foods. Transgenic animals can also secrete in their milk, proteins such as blood clotting factors needed by patients of haemophilia.
Caseins, being easily digestible are quite sensitive to plasmin, a serine protease occurring naturally in milk and also plasminogen. Thus, β-casein the most abundant casein in ruminant milk undergoes limited proteolysis by plasmin. This can be disadvantageous as casein proteolysis decreases the curd yield in cheese and can induce organoleptic defects and gelation of UHT milk. A milk enriched with specific inhibitor of either plasmin or plasminogen activator would therefore be alternative for the process industry.

Several human proteins that are of high value, low volume and therapeutic have been expressed in milk of domestic animals with success. The major advantage of transgenic technology is that these proteins can be produced at a very low cost. Economic comparison of production costs of human tissue plasminogen activator (htPA) through bacterial fermentation, mammalian cell culture and cow transgenic technology estimates the cost per gram of htPA to be 20000, 10000 and 10 US dollars respectively. Two proteins, human antitrypsin and human antithrombin II have been purified from milk of transgenic ruminants. Human antithrombin III, a plasma protein that helps prevent harmful blood clotting is also being tested.

5. Humanisation of bovine milk

It is said that mother's breast milk is the ultimate designer food for babies. However, due to varying reasons, a number of infants are fed formulas based on bovine milk. The composition of these formulas could be greatly improved to suit the needs of the infant by incorporating ingredients that resemble those of human milk, thereby 'humanising' the bovine milk.

The level of lactoferrin (LF), the iron-binding protein has antimicrobial properties and may also mediate some effects of inflammation and have a role in regulating various components of the immune system in human milk is about one g/l (human colostrum – 7 g/l). The levels of LF in cow milk being only about one tenth of that in human milk, this has prompted researchers to design human milk replacement formulas. The human LF (hLF) gene has already been expressed at low levels (0.1 to 36 mg/ml) in the milk of transgenic mice and a transgenic bull that carries the gene for hLF has been produced.

Human milk contains 0.4 g/L of lysozyme (LZ), an enzyme that provides antibacterial activity in human milk. Active human LZ (hLZ) has been produced in the milk of transgenic mice at the concentrations of 0.78 g/L. On the processing front, the expression of LZ in milk results in the reduction of rennet clotting time and greater gel strength in the clot. A double transgenic cow that co-expresses both hLF and hLZ in milk may also reduce the incidence of intra-mammary infection or mastitis.

Yet another application of transgenic technology could be to produce the human lipase, which is stimulated by bile salt in the milk of bovines. The lipase thus produced could be used as a constituent of formulas to increase the digestibility of lipids especially in premature infants who have low lipase activity. Several children are allergic to cow’s milk, owing to the presence of β-lg, which is not found in human milk. Elimination of this protein by knocking out β-lg gene from cow’s milk is unlikely to have any detrimental effects, on either cow or human formula, and might actually overcome many of the major allergy problems associated with cow’s milk.

6. Additional advantages
The use of molecular biology to reduce the presence of pathogenic organisms such as salmonella and lysteria in milk is a potentially beneficial prospect. It might be possible to produce specific antibodies in the mammary gland that are capable of preventing mastitis infection or those that aid in preventing human diseases. Mice that produce milk with 33% more total solids (40-50% TS) and 17% less lactose than normal control mice have been generated by transgene. Due to a decrease in lactose synthetase activity, less lactose is being produced and less water is being transferred into milk causing a reduction in milk volume. So it appears that the same amounts of total milk fat and protein are being produced in a lesser total milk volume. If this technology could be translated to dairy animals, milk that contains 6.5% protein, 7% fat, 2.5% lactose and 50% less water is not an improbable accomplishment. This would mean a direct economic benefit in terms of 50% reduction in the cost of shipping milk. In addition, since the cow would be producing one half her normal volume of milk there would be less stress on the cow and on her udder. From the processing point of view, after removal of fat from this type of milk, a skim milk having twice protein content and have half the lactose content of normal milk could be produced. This type of milk would also make it easier to produce low lactose or lactose free dairy products. The concentrated milk should lead to better product yields from the same amount of initial input. The lowering of milk volume and lactose content will reduce the total whey output produced during processing. The reduction of stress on the mammary gland of the cow and the more viscous milk may also decrease the susceptibility to obtaining mastitis infection. Organisms that cause mastitis use lactose as their energy source and since lactose would be reduced in the system there would be a decrease in the available food source for these bacteria.

7. Conclusion

Despite all efforts and positive results, the status of biotechnologically derived foods is not very advanced. Hi-tech milk processing may be more acceptable to consumers than transgenesis for altering milk composition. Acceptability will depend ultimately on animal welfare, demonstrable safety of the product, enhanced health properties of the product and increased profitability as compared with conventional practices. Various ethical, legal and social aspects of biotechnological research need to be addressed before we would see designer transgenic herds similar to the organic herds that thrive in the current economic and social climate.

8. Selected Reading


Composite Dairy Foods: Prospects & Issues

Ashish Kumar Singh and P. Narender Raju
Dairy Technology Division

1. Introduction

Food industry always looks for “Product diversification” for its long-term sustainability. Companies engaged in R & D related to improvement of the quality of existing products and introduction of newer variants of foods. Diversification will not succeed until it is of commercial significance and therefore industry must keep an eye on fast changing consumer attitude and behaviour. In our country there has been paradigm shift towards in-house R & D among food and dairy processing industries. Our more than 1.2 billion population with great cultural diversity that also reflects in the food choices offer newer opportunities for launching of innovative foods.

Increasing demand for foods with better nutritional status and also their ability to deliver bioactive components with certain therapeutic attributes has mandated the designing of novel foods. In India, product designers also consider affordability of different economic strata. Product development is a complex phenomenon that requires a great deal on selection of ingredients, understanding of macromolecule interactions in synergistic or antagonistic way and its impact on quality & stability of developed product.

Using unrelated food groups for the development of new product has been practiced since time immemorial; however it was solely driven by deriving a new flavour. In modern age such experimentation are aimed at complementation for organoleptic, functional and health virtues. It has led to the concept of “composite foods”. Although, there is no well defined category exists as “composite food”, but the term could be used for any formulated food which contain appreciable proportion of two different food groups. There could be numerous such possibilities exists in formulation, however composite dairy foods based on milk-cereal and milk-fruits & vegetable combinations are more popular. The current article focus on technological parameters, issues and prospects related to composite dairy foods.

2. Composite dairy foods

Milk is considered as “complete foods” because of ample of nutrients as well as physiologically active components it possess, but milk too lack certain critical micronutrients (iron, copper and certain vitamins) and fiber. Moreover, using milk alone may not enhance acceptability developed products. Therefore, there is obvious need for supplementing milk with necessary micronutrients as well as health promoting components form suitable sources. As regards the supplementary sources of nutrients and health factors for the milk several options including fruits & vegetables, cereals, millets, legumes and certain oilseed crops, could be adopted. Likewise milk ingredients are essential components in formulation of wide range of processed foods, most of them fall in the category of functional and or health foods.

Several examples of composite dairy foods exist in different parts of the world and some of the products have already been commercialized. However, there is not much scientific perspective related to the complementary or supplementary role of such formulations exists. The addition of non-dairy components in milk is mainly target improvement in sensory quality in terms of flavour, texture and mouthfeel, however nowadays such
formulations in addition to organoleptic improvement also target enhanced health attributes. Milk ingredients mostly in the form of powders have been used by other segments of food industry for nutritional enhancement and better functionality of developed products.

3. Promising candidates for composite dairy foods formulations

Cereals including wheat and rice are the major source of calories, proteins and other micronutrients in our diet. With advancement in milling and other related primary processing unit operations nowadays these commodities are consumed in refined form that lack several micronutrients and fibers. On the other hand a number of food preparations are prepared by combining cereal grains/flour with milk. It not only enhances the palatability of these commodities but also improves the nutritional value. The classical examples of milk-cereal based “Composite Dairy Foods” are kheer and malted milk foods. Re-emergence of coarse cereals and millet crops owing to multifaceted benefits associated with their cultivation and consumption has generated a great interest among food formulators. India is the largest producer and consumer of millets including pearl millet, finger millet, foxtail millet, kodo millet, little millet etc. Coarse cereals and millets are relatively rich in proteins, minerals and vitamins in comparison to conventional cereal crops. The nutritional significance of these crops lies in their richness in micronutrients like calcium, iron, phosphorus, zinc, vitamins and sulphur containing amino acids (Rai et al., 2008). The functional properties of millet starch are comparable to other cereals. The higher proportion of non-starchy polysaccharides, dietary fiber and low glycemic index make them an ideal ingredient in many food formulations meant for diabetic and persons with coronary heart disease. These contain polyphenols, lignans, flavonoids, phytosterols and (Hulse et al., 1980; Dykes & Rooney, 2006; Awika et. al., 2004). Incorporating barley in a healthy diet may be effective in lowering plasma total cholesterol and LDL cholesterol (Behall et al., 2004). Barley and Oat are two minor cereal crops endowed with certain unique functionality that can be exploited in formulation of several products (Kaur et al., 2012).

Presence of thick pericarp, pigments, certain phenolics, anti-nutrients, and absence of primary processing equipments are the major hurdles in widespread consumption of these crops. The processing mediated inactivation of anti-nutrients could be applied for enhanced nutrient availability and product development. Moreover, processing mediated interventions further inactivates the anti-nutrients like phytate and lipase and enhances the shelf-life of resultant primary processed products.

India is among the top most producer of fruits and vegetables in the world and lack of poor emphasis on post harvest management practices lead to 30-40% losses. Utilization of fruits and vegetables along with milk in new product development could be an attractive alternative in enhancing the rate of value addition. Addition of fruits and vegetables during manufacture of beverages, ice cream, yoghurt, yoghurt drinks and in certain traditional products such as shrikhand, is widely practiced to enhance their acceptability. Phytochemicals present in fruits and vegetables have generated immense interests among R & D personnel for their utilization in food products with specific health attributes. Indigenous as well as exotic plant materials could be suitable candidates for such formulations.

Among the dairy products whey and whey nutrients exhibit excellent opportunity for composite dairy foods. Whey proteins are one of the highest quality food proteins with a high PER (3.6), biological value (104), NPU (95) and highest Protein Digestibility Corrected Amino Acid Score (PDCAAS) score that make then an ideal protein source for
fortification of wide range of food stuffs. Dietary whey proteins have a number of putative, biological effects when ingested (Horton, 1995). Whey proteins are rich source of all essential amino-acids specially the sulphur containing amino-acids i.e. cysteine and methionine which make them superior to meat, soy and casein. Tryptophan, which acts as building block for niacin, is present in high amount in whey proteins. Liquid whey can be utilized as such or in concentrated form as whey powder for the production of higher value added products like lactose, whey protein concentrates (WPC) or whey protein isolates (WPI). Dairy ingredients also possess unique functional characteristics such as water binding, gelation, emulsification, whipping colour and flavour formation.

4. Issues related to development of composite dairy foods

Although, addition of non-dairy ingredients looks quite simple, but it is more complex and a clear understanding of resultant phenomenon and strategies to overcome adversities is required to make the products stable.

- Many technological problems may occur upon addition of cereals/millets/fruits & vegetables into milk, mainly due to the numerous reactions of non-dairy components with milk molecules. The addition of cereals/fruits & vegetables in milk destabilizes the milk proteins by disturbing the salt balance. It may also lower the pH of the system that led to coagulation of milk proteins during heating.
- Some of the micronutrients like iron promote the oxidation of fat rich dairy products that may adversely affect flavour and storage stability of composite products.
- The interaction of polyphenols present in non-dairy materials with milk proteins has been investigated by number of researchers and form insoluble complex. It not only affects the bioavailability of proteins but also affect the stability of mix (Hurrel et al., 1982).
- Addition of starchy materials causes rapid increase the viscosity of composite mix, which may affect the thermal processing and mechanization. Addition of starch may also affect the heat coagulation time (HCT) of milk (Nayak et al., 2004). It also limits the level of starch containing materials such as cereals, millets, legumes, tuber crops etc. in milk. Higher levels could results in gelled products, which is prone to retrogradation during refrigerated storage.
- Interaction of milk constituents with pectin presents in fruits may increase the viscosity of the mix (Jelen et al., 1987), which is negatively evaluated in liquid products, but desirable in gelled products. Pectin do assists in stabilization of milk proteins in fruit and or acidified beverages.
- Naturally occurring colouring pigments in fruits and vegetables produce off-colour products if added in lower amounts. Loss of colouring pigments may also occur during thermal processing. These reactions could be reflected in changes in texture, colour, sedimentation, flavour and/or the functional properties of the product. It could also lead to failure of product in the market place as suggested by the figures of the product recalled every year. However, by adopting appropriate formulation and technological interventions these adverse effects can be avoided.

5. Prospective composite dairy foods

5.1 Malted and complementary foods

Malted cereal milk foods are also widely manufactured in the world and finds a good market. Process of malting activate the native enzymes present in cereals, minimize the
level of antinutrients and render them more suitable for value addition. Since majority of nutrients are present in pre-digested form, hence can serve as ideal ingredients for speciality foods meant for infants, weaning and aged persons. At present the available malted milk products commercially available are based on barley or wheat solids, however there is substantial scope for utilizing the malt form other coarse cereals/millets. Many workers reported the production of millet malt mixes incorporating other malted grains and ingredients, these dry mixes on reconstitution in water and or milk produced malt beverages (Malleshi and Desikachar, 1982; Kshirsagar et al., 1994)

Inadequate nutrition usually provided through complementary foods leads to problems associated with malnutrition across the globe. Traditional infant-feeding practiced, in countries like India, is usually cereal based. For the manufacture of weaning foods cereal grains are often germinated, fermented, processed and cooked in various ways to improve digestibility, and mixed with oils or animal products to enhance their nutritional profile (Haidara, 1990), however most of these complementary foods are reported to be less energy dense and less safer for children. Cereals in combination with milk solids are generally used for the preparation of weaning foods. On the basis of scientific literatures available it could be inferred that the inclusion of traditional technologies, such as malting and popping of cereals and legumes, for the preparation of weaning and supplementary foods. The process of malting/germination minimizes the levels of anti-nutrients and enhances the digestibility. It further assists in incorporation of higher proportion of flours in formulation without increasing the viscosity. Process of roasting may further improve flavour and nutritional characteristics of germinated flour, but it may adversely affect the available lysine content in resultant product. Modi (2009) optimized a process for the development of low cost complementary food by using whey-skim milk blend as base and incorporation of germinated pearl millet, barley malt and corn flour. The mix could be either spray or tray drier to yield a powder, which was further blended with sugar and flavouring. The finished product meet all the specifications laid down in FSSA for milk-cereal based complementary food.

5.2 Milk-cereal based fermented foods

Fermented cereal-yoghurt mixtures play an important role in the diets of many people in the Middle East, Asia, Africa and some parts of Europe. *Tarhana* is a traditional fermented food product of Turkey. *Tarhana* is prepared by mixing yoghurt, wheat flour yeast and a variety of vegetables and spices followed by fermentation for 1-7 days. After fermentation the mixture is sun dried and ground. *Tarhana* has an acidic and sour taste with a yeasty flavour and is used for soup making.

*Rabadi* is a fermented indigenous food of India especially useful for low and average income rural people. It is popular is North-Western semi-arid regions of India and can be prepared by mixing and fermenting flour of wheat, pearl millet, barley or maize with buttermilk in summer months at room temperature (40-45°C) for 4-6 h. The fermented product is boiled, salted to taste, cooled and consumed. It is a lactic acid fermented food in which lactose undergoes acid fermentation naturally and readily (Gupta, 1989). Work carried out at NDRI showed that lactobacilli could be effective in reducing the levels of anti-nutrients i.e. phytic acid, total phenols and tannins and improving the antioxidant potential as well as in-vitro availability of minerals from a milk-pearl millet composite.

Cereals/millets have potential to be incorporated in probiotic dairy foods formulation because of their richness in fiber, oligosaccharides, free amino acids and certain minerals which promote the growth of probiotic bacteria. Human-derived strains of *L. reuteri*, *L. plantarum*, *L. acidophilus*, and a *L. fermentum* strain isolated from cereals when cultured
in malt, barley, and wheat extracts exhibited better cell growth in malt medium than in barley and wheat extracts due to the higher proportion of maltose, sucrose, glucose, and fructose (Charalampopoulos and Pandiella, 2010). *Lactobacillus plantarum* Lp-9, an indigenous probiotic strain also appeared promising in formulation of barley-milk based probiotic beverage.

5.3 Milk-coarse cereals/ millet based convenience foods

Breakfast cereals are grain foods, usually in a precooked or ready-to-eat (RTE) form, usually consumed with milk or cream during breakfast and often sweetened with sugar syrup or honey. Roasting, popping and puffing are the traditional processes normally used to produce breakfast cereals. Millets have seldom been used in preparation of such products, though utilization of millets in their formulation will certainly enhance nutritional quality. It is another approach for increasing the level of dietary fibres, micronutrients and nutraceutical concentrations in RTE products. Extrusion cooking is another emerging technology for the manufacture of RTE foods, snacks and porridges. The process of extrusion pre-gelatinizes starch, denatures proteins and inactivates various anti-nutrients, thus improving the digestibility and bio-availability. Meena (2010) optimized the formulation and processing conditions for the preparation of milk protein-enriched extruded snacks based on pearl millet. Application of germinated pearl millet flour in combination with milk protein sources and corn flour resulted in low fat, high protein and fiber products. Extrusion processing is also employed for the pre-gelatinization of pearl millet grains followed by grinding and mixing with whey powder and milk solids to yield a nutritionally rich porridge. The process of extrusion reduced the phytates and total phenols by 50-68% and 30-40% respectively. Extruded barley and pearl millet were formulated with whey powder and skim milk powder (SMP) to develop low cost weaning food.

5.4 Utilization of milk ingredients in gluten free bakery products

Millet flour can also be used for the manufacture of various bakery products in combination with wheat flour. Lack of gluten in millets limit their level in such formulations, however up to 50% incorporation in formulation of biscuits has been reported. Suitable primary processing interventions like steeping, pearling and germination may assist in enhancing the level of millet flour in bakery formulations. Millet-based products may also be an ideal alternative for patients with celiac disease. The incorporation of milk proteins, especially in the form of whey proteins help in development of exclusively millet-based gluten free bakery products. Pre-treatments, particle size of flours and other ingredients in formulation affect the quality characteristics of millet based bakery products. A process technology for the manufacture of whey protein and iron fortified pearl millet biscuit is developed. The developed biscuit has a shelf-life of more than 6 months. The validation studies in animal models i.e. rat, indicated that consumption of whey protein enriched and iron fortified biscuits increased the haemoglobin and serum ferritin level in anaemic as well normal rats (Gayathri. 2011).

6. Milk-fruits and vegetable based composite dairy foods

6.1 Milk-fruit beverages

Milk based beverages have emerged as one of the most promising segment among value added dairy products. Dairy industry must exploit the opportunities to keep pace with the changing pattern of product consumption and increasing demand of health foods. Apart from traditional milk beverages, there is a wide range of fruit based dairy beverages that could be added to the product portfolio. Addition of fruit pulp or juice causes disturbance
of salt balance and precipitation of milk proteins due to low pH. Stabilization of such acidified milk beverages is quite challenging task. Application of high methoxyl (HM) pectin is effective in preventing the coagulation of milk proteins during processing and further sedimentation during storage. HM pectin absorbs onto the micellar caseins mediated by the charged blocks of the pectin molecule, while the uncharged domains form loops that extend into the solution. Among the fruits strawberry, citrus, mango, pineapple, cherry, blueberry and banana are preferred for blending with milk. Proportion of these fruits may vary from 5-20 percent. The addition of fruit components have been reported to enhance the micronutrient status of resultant products. Novel processing interventions such as pulsed electric field (PEF) and high pressure processing could be applied to deliver a shelf-stable product.

6.2 Utilization of fruits in fermented dairy products

At present around 7% of the milk produced in India is utilized for the manufacture of fermented dairy products. Recently fruit based yoghurt has also been introduced in Indian market which is also becoming popular day-by-day. Addition of fruits as pulp or juice in dahi or yoghurt improves the quality and acceptability of resultant products. For the manufacture of fruit yoghurt, fruit pulp or juice is used typically in the range of 4-20%. Addition of mango pulp above 4.0 percent was reported to adversely affect the delicate yoghurt flavour and also the body and texture despite using homogenization pressure. However homogenization pressure up to 200 bars may help in producing yoghurt with smoother consistency and also minimize the whey separation. Fruit dahi was prepared by suing mango, banana, pineapple and strawberry @ 6, 8, 6 and 4 percent levels and among them mango dahi was found to be most acceptable.

Shrikhand, a popular protein and sugar-rich fermented dairy product is also blended traditionally with mango pulp to prepare ‘Amrakahnd’. Published literature on probiotic fruit and vegetable containing fermented dairy products showed that fruit and vegetable constituents not only assist in development of desirable sensory attributes but also enhance the survivability of probiotic cultures. Most probably the cell wall constituents or polysaccharides present in fruit pulp act as prebiotic substance and also as encapsulating agent.

6.3 Application of milk ingredients in fruit and vegetable based products

Milk ingredients such as milk powder, casein & caseinates and whey protein preparations may find novel application in various categories of fruit and vegetables based products. Development of “Functional fruit beverages” is an attractive possibility to utilize milk nutrients for human beings. Among the milk proteins whey proteins could be utilized as ideal protein source for fortification of fruit juices, beverages and specialty foods. Whey proteins have unique amino-acid profile, rich in branched-chain as well as sulphur containing amino acids. Besides it, whey proteins are also rich in cysteine that is required for the biosynthesis of glutathione, a tri-peptide and natural anti-oxidant. Whey protein may find applications in both dairy type as well as fruit based acidic drinks. Some of the technological advantages associated with utilization of whey proteins in beverages include, their solubility over a broad range of pH (3-8), they have bland flavour so their inclusions in formulation do not cause cheesiness, saltiness, in developed products. Whey protein possesses excellent buffering capacity that is advantageous particularly in probiotic drinks where it help in survival of “live” bacteria in stomach. Moreover, the addition of whey protein improves the viscosity of beverages; hence they can substitute stabilizers in beverages and therefore enhance the “mouthfeel”.
7. Conclusion

Majority of health foods attract little effective demand in Indian market and have to face competitions from established brands. The recent growth and upward trend of Indian food market offer new opportunities for the development of such health foods by judicious blend of milk/milk constituents and cereals, into convenient, long-life form with proven health benefits to consumers.

Milk nutrients are considered essential components of diets among all age groups. Likewise, many agricultural commodities including cereals, millets, legumes, are important constituents of the diets of poor across the globe. There is readymade demand for quality weaning foods based on milk-cereal blend. Experience with such food products which are adequately formulated and processed in meeting the nutritional needs of infants and children, is quite encouraging. Hence still there is requirement of milk-cereal based weaning or complementary foods that can assist us in alleviating the problem of malnutrition and hunger. Likewise, milk-cereal based probiotic fermented products may also have a lot of commercial significance.

8. Suggested Readings


Haidara, M., (1990). L’Utilisation du Me´lange de Farine Mil/Nie´be´ dans L’Alimentation Journalie`re au Mali. La Cellule de Technologie Cerealie`re (SRCVO/IER), Le Service de Nutrition, INTSORMIL, Bamako, Mali


1. Introduction

Packaging is an external means of preservation of food during storage, transportation and distribution. Hence, it forms an integral part of the product manufacturing process. Food packaging performs four major disparate functions such as containment, protection, convenience and communication. In pursuit of achieving these goals, many materials have been discovered by man for use as food packaging materials. Owing to increasing urban lifestyle and global population trends, the demand for packaged food including frozen and ready-to-eat has witnessed a significant surge in demand in recent times. With the supply of food from across the borders of a country, the packaging industry has been focusing on development of solutions that provide maximum food security while maintaining nutritional value at competitive prices. Global advanced packaging technology market is witnessing a high growth due to the increase in demand for convenience food such as ready-to-cook meal, stricter food safety regulations, and demand for sustainable packaging. Growing health awareness among consumers, food wastage, and manufacturers’ concern for longer shelf life of the food products and supply chain inefficiencies are the other factors fueling the growth of the market. Further, the consumers’ confidence with regards to packaged food, organized food safety regulations, threat from bioterrorism, and increased applications in ready-to-eat meals and frozen foods have created a huge opportunity for advanced packaging technology market. The global advanced packaging technologies viz. modified atmosphere packaging, active packaging and intelligent packaging is estimated to grow at a CAGR of 8.2% from 2010 to 2015. Hence, packaging technology is to be of strategic importance to a company, as it can be a key to competitive advantage in the food industry. This can be achieved by catering to the needs and wants of the end user, opening up new distribution channels, providing a better quality of presentation, enabling lower costs, increasing margins, enhancing product/brand differentiation and improving the logistics service to customers. Marketing mix is an essential business tool and is crucial when determining a product or brand’s unique selling proposition (USP). The challenge of any business is to have a sustainable and profitable venture by tackling the marketing mix. In the present article, the role of packaging and novel packaging systems in improving sales and profit of a business venture is presented.

2. Marketing mix

Marketing is defined as “the process which identifies, anticipates and satisfies customers' requirements profitably”. In other words, it is finding out what products or a services customer want, either now or in the future, and provides those products or services to them at a price which leaves a profit for the business. The market-driven businesses will change the product or service to suit the customer. Marketing mix is the set of marketing tools that a firm uses to pursue its long-term and short-term marketing objectives in the target market. The idea of the marketing mix is the same idea as preparing a cake from a cake mix. A baker will alter the propositions of ingredients in a cake depending on the
type of cake the consumer wishes to have. The proportions in the marketing mix can be altered in the same way and differ from the product to product. Hence, it includes all the key activities which are used in marketing a firm’s products. The marketing-mix decisions influence the trade channels as well as final consumers. These tools are traditionally classified into four broad groups referred to as the four Ps: Price, Promotion, Place and Product. Some of the recent literature reveals that it is classified into seven Ps viz. price, product, promotion, place, process, physical evidence and people. Although the 7Ps concept is comprehensive it is also complicated. Packaging, one of the key elements of marketing mix associated with product, can improve the profits of a business, if the actions are directed towards meeting customer’s wants and demand. The consumer needs and wants of packaging, an important component of marketing mix, are given in Table 1. Packaging plays an important role as a medium in the marketing mix, in promotion campaigns, as a pricing criterion, in defining the character of new products, as a setter of trends and as an instrument to create brand identity and shelf impact in all product groups.

<table>
<thead>
<tr>
<th>Table 1. Consumer Needs and Wants of Packaging</th>
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<td>Quality</td>
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<td>Information</td>
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<td>Convenience</td>
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<td>Product availability</td>
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<td>Variety</td>
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<td>Health</td>
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3. **Profit drivers of a business enterprise**

In order to achieve consistent profitability, a business enterprise should know the critical factors that drive the profitability and work relentlessly on those areas of the business. For most business ventures including food industries, there are four major profit drivers: (a) price, (b) variable costs, typically represented by cost of sales, (c) fixed costs or overheads, and (d) sales volumes. All the possible strategies, tactics, devices, new technologies and products that can be devised to increase profits, fall into one of these four drivers. It means that increase in sales price, increase in sales volume, reduction in unit costs and reduction in overheads per unit of sale. In fact, price will always have an impact that is two or three times greater than any of the other drivers. The reason for this is quite straightforward: an increase in price has the greatest impact because every additional rupee goes straight to profit. By comparison, an increase in sales volume will be accompanied by an increase in variable costs, so the gain will be smaller. A decrease in variable costs will increase the margin but will not increase overall revenue. Finally, a reduction in fixed costs has no impact on revenue and therefore will always have the smallest impact of all.
4. Packaging and packaging systems role in food product sales and profit

Packaging for fast moving consumer goods has been considered as part of the food retail marketing mix and thus closely affects all the other marketing variables i.e. product, price, promotion and place. Packaging also plays a vital role in food marketing representing a significant key to a brand’s success or mere survival in a highly competitive market place. Packaging innovation and design are in front line of competition between the brands of both major retailers and product manufacturers. On an individual product/brand basis, success is dependent on the product manufacturer’s rapid innovative response to major trends. One of the most effective ways to respond is through distinctive packaging, and this has become one key factor in the success of a brand. Brand differentiation can be enhanced by innovative packaging designs that confer aesthetic and/or functional attributes. Packaging also plays an important supporting role in projecting the image of the retailer to gain competitive advantage. The general purpose of the image of retailer’s own brands is to support the overall message such as high quality, healthy eating, freshness, environmentally aware and value for money. Factors that influence the retail acceptability of a product as affected by packaging are given in Table 2.

<table>
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<th>Table 2. Retail acceptability of a product as affected by packaging</th>
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<td><strong>Sales appeal to target customer</strong></td>
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<td>Retail competition</td>
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<td>Retail environment</td>
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<tr>
<td>Brand competition</td>
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<td>Brand image/positioning</td>
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<td>Brand persona</td>
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<td>Brand impact/differentiation</td>
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<td>Brand promotion</td>
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<td>Brand communication/presentation</td>
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<td>Consumer and brand protection</td>
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<tr>
<td>Retail customer service</td>
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<td>Retailer’s margin</td>
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With the increasing urban lifestyle and global population trends, the demand for packaged food has witnessed a significant rise in demand in recent times. With supply of exotic fruits and vegetables, meat products and frozen foods surpassing geographical boundaries, the packaging industry has been focusing on development of solutions that
provide maximum food security while maintaining nutritional value at competitive prices. The food and beverage packaging has dramatically shifted from traditional to advanced packaging. Traditional packaging only addresses issues related to protection from external factors. However, advanced packaging interacts internally (active packaging) and externally (intelligent packaging) with the environment and enhances the visual appeal of the products. New technologies such as intelligent packaging, smart packaging, active, and modified atmosphere packaging are replacing traditional methods such as canning. Active and smart packaging technology offers tremendous potential to fulfill the growing demand of food safety in various applications which include dairy products, meat and poultry, ready-to-eat meal segment. In active packaging, oxygen scavengers and moisture absorbers form the two largest product segments. Both are estimated to grow at a CAGR of 8% and 11.9% respectively. In 2010, the active and intelligent packaging technology held the highest growth rate, estimated at a CAGR of 10.5% and 12.1%, respectively from 2010 to 2015. Modified atmosphere technology accounted for largest share (approximately 54%) of the total market in advanced packaging technologies. Another emerging technology in the market is nanotechnology. Nanomaterials have various applications; both in active and intelligent packaging. In active packaging, the nanostructures that can enhance the vapor permeability of plastics are used. The nanosensors categorized under intelligent packaging can help in detecting pathogens, toxins, and chemicals. With nanosensors incorporated inside the packaging, the consumer can easily know the status of food inside, which means these sensors can inform the consumers about the food’s freshness level and nutrition status.

5. Conclusion

Owing to increasing urban lifestyle and global population trends, growing health awareness among consumers and organized food safety regulations, the demand for safe and healthy packaged food has been increasing. The challenge of any food enterprise is to remain sustainable and profitable by tackling the different elements of marketing mix. Appropriate use of novel packaging designs and techniques, aimed at meeting the requirements of all the stakeholders, could be an important option for the industry to sustain and bag profits.
Microencapsulation: Strategy to Enhance Stability of Probiotics

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Dairy Technology Division

1. Introduction

The term ‘probiotic’ refers to live microbial feed supplements that beneficially affect the host by improving its intestinal microbial balance. There are many reports on the potential role of probiotics on the improvement of lactose assimilation, food digestibility, hypercholesterolemia, and immune response, or on the prevention of intestinal infections, vaginal infections, cancer, food allergies, and constipation. Researchers have incorporated probiotics in variety of food products, including soft, semi-hard and hard cheeses, ice cream, milk powder, fruit products, frozen dairy desserts as well as oat-based fermented products.

2. Challenges in maintaining stability

Survival of probiotics in products is affected in dairy and fermented products by a range of factors including processing conditions, pH, acidification during storage in fermented products, hydrogen peroxide production, oxygen toxicity (due to oxygen permeation through packaging), storage temperatures, stability in dried or frozen form, poor growth in milk, lack of proteases to break down milk protein to simpler nitrogenous substances and compatibility with traditional starter culture during fermentation (Shah, 2000). Oxygen tolerance plays a major role in the poor survival of probiotic bacteria. Some strains of probiotics, especially \textit{Bifidobacterium} sp., lack the ability to survive gastrointestinal conditions. The increasing demand for probiotics and the new food markets where probiotics are introduced challenges the industry to produce high quantities of probiotic cultures in a viable and stable form and in sufficient number. Thus, it is important to develop methods to maintain and/or to promote their viability throughout the product shelf life.

Probiotics play multiple roles in dairy fermentations, thereby resulting in products with the organoleptic properties desired by the consumer and also in improved nutritional and therapeutic value of the food. Some of these roles are: 1- preservation of milk by generation of lactic acid and/or other antimicrobial compounds like bacteriocins, 2- production of flavour compounds, 3- reducing off flavours as in metabolizing the compounds that produce the beany flavour in soy products, 4- synthesis of useful metabolites such as extracellular polysaccharides, vitamins, free amino acids, 5- special therapeutic or prophylactic properties against intestinal disorders, cancer, 6- control of serum cholesterol levels). In order to exert all these positive traits in a product these organisms should remain metabolically stable and active, surviving passage through the upper digestive tract in large numbers sufficient enough to produce beneficial effects when in the host intestines. Adequate numbers of viable cells, namely the ‘therapeutic minimum’, need to be regularly consumed in order to transfer the probiotic effect to consumers. Survival of these bacteria during the product shelf life until being consumed is, therefore, an important consideration.
3. Challenges and strategies

From a technological standpoint, the challenges in the development of stable probiotic starters for food applications are selection of an appropriate strain, preparation of inoculums, enabling growth and/or survival during processing, assessing the interactions among probiotic and other starter organisms and taking care of the effect of probiotic strains on sensory properties of the product. The most crucial characteristics for a probiotic strain are acid and bile resistance and adherence to intestinal mucosa, in order to exert their health promoting effects. Several approaches are being explored to increase viability of probiotic bacteria in commercial and experimented products, including selection of acid and bile resistant strains, control of over-acidification of dairy products, and the addition of cysteine or an oxygen scavenger such as ascorbic acid, use of oxygen impermeable containers, microencapsulation, two-step fermentation, stress adaptation, and incorporation of micronutrients such as peptides and amino acids.

4. Microencapsulation

Microencapsulation is a process in which cells are retained within an encapsulating membrane to reduce cell injury or cell loss resulting in sealed capsules that can release their contents at controlled rates under specific conditions. The capsules may be of different shapes and can range in size from submicron to several millimetres in size. Micro-encapsulation has many advantages over immobilisation/entrapment techniques. The microcapsule is composed of a semi permeable, spherical, thin and strong membranous wall. Therefore, the bacterial cells are retained within the microcapsules with the nutrients and metabolites diffusing through the semi permeable membrane easily. Several food-based substances have been microencapsulated, such as acidulants, amino acids, antimicrobials, bases, colorants, edible oils, flavor, enzymes, microorganisms, flavor enhancer leavening agents, minerals, sugars, salts and vitamins. Microencapsulation of probiotics offers a potential way of improving their survival during manufacture and storage of functional dairy products and during GIT transition (Picot and Lacroix, 2004). The most widely used matrix for microencapsulation is alginate, though gelatine and vegetable gum have also been reported to provide protection. Alginate beads reportedly increase the survival of probiotics by up to 80-95% (Krasaekoopt et al., 2003). There are a number of techniques being used but the most common techniques are emulsion, extrusion and spray drying.

4.1 Emulsification

This technique is based on the relationship between the discontinuous and continuous phases. The probiotics are mixed into a solution of alginate (discontinuous phase) and dropped into vegetable oil (continuous phase- soybean, sunflower, canola or corn) containing a suitable emulsifier (Tween 80) and surfactant (sodium lauryl sulfate) to form beads. The latter are harvested by filtration. This technique is easy to scale up for large-scale production and provides both encapsulated and entrapped core materials. Adversely, the residual oil, emulsifier and surfactant in the encapsulating material can affect the growth of live probiotics and interact with food components. In addition, the residual oil may damage the organoleptic properties and texture of the food products. It is also not suitable for low fat products. There are numerous reports of the application of this method for microencapsulation of probiotics. Work conducted at NDRI, Karnal reports the effect of microencapsulation using sodium alginate and starch on the tolerance of probiotic Lactobacillus paracasei S233 (Babu et al., 2009) and L. acidophilus LA1 (Sabikhi et al.,
2010) by emulsion. The organisms survived well in the protected form at high temperatures, high salt concentrations and at simulated conditions of gastric pH and at high bile salt concentrations.

4.2 Extrusion

This involves extrusion of solutions containing an emulsion core and coating material through a nozzle at high pressure to produce capsules. It is reported mainly on a laboratory scale, using simple devices such as syringes. In this method, the bacterial cells and polymer suspension is extruded through a needle or a nozzle, generating spherical droplets that fall into a hardening solution. Survival percentage with this technique is about 85-90%. However the major disadvantage is large bead size formed which is not desirable for food applications and is difficult to scale up.

4.3 Scaling up of technologies

The choice of the encapsulation techniques depend mainly on the desired size and, size distribution, productivity, capital and operating costs. The criteria of selection for industrial scale are low capital and operating costs and small bead size (<1000 μm).

4.3.1 Use of air atomizers for alginate gel encapsulation

Air atomisation is well-established spraying system, which is used in numerous industries such as medicine, food, chemical processing, and agriculture for production of fine powders. The conditions employed are mild and result in small beads. The process is continuous and consumes less power (Teunou and Poncelet, 2004). In continuation of the earlier study, Sabikhi *et al.* (2011) investigated the application of the air atomization method for co-encapsulation of probiotic *L. acidophilus* NCDC15 with the prebiotic inulin. The results indicated that the organisms survived better and that the product could be useful in synbiotic food applications.

4.3.2 Spray drying

Spray drying is an alternative low cost encapsulating method that can be conducted in a large scale. By the careful selection of processing conditions, spray drying can give high survival of encapsulated probiotic organisms. The addition of protectants to the media prior to drying is a common approach to improve probiotic performance in food systems. The incorporation of thermoprotectants such as trehalose, skim milk powder and/or adonitol, granular starch and growth-promoting factors including prebiotics have been employed in efforts to improve culture viability during drying, storage and/or gastric transit. The optimal conditions that have to be fulfilled in order to protect probiotics during spray drying and storage are a) type of strain and its tolerance to stress, b) carrier, c) drying temperature, and d) time of exposure to heat, osmotic, oxidative, and mechanical stresses. Salaria (2011) attempted spray drying as an encapsulating technique for probiotic *L. acidophilus* LA1 using appropriate protectants and found that ~ 87% of the cell mass survived the spray drying process. Storage at ambient temperatures registered no significant cell loss after a month.

5. Conclusion

The health properties of probiotic strains should be preserved in the products sold at the time of consumption, if probiotic products are to gain popularity among consumers. To guarantee this, many important variables must be considered by the dairy industry. Microencapsulation is a very effective method to achieve the dual goals of survival of
sufficient numbers of probiotic cells throughout the shelf life of the product as well as during gastro-intestinal transit.

6. Selected Reading


Salaria A (2011) Preparation of probiotic adjunct for infant food formulation. Ph.D. thesis submitted to NDRI (Deemed University), Karnal


1. Fermented milks - definition

Fermented foods are of great significance since they provide and preserve vast quantities of nutritious foods in a wide diversity of flavors, aromas and textures, which enrich the human diet. Over 3500 traditional, fermented foods exist worldwide. The International Dairy Federation published general standards of identity for fermented milks that could be briefly defined as follows: Fermented milks are prepared from milk and/or milk products (e.g., any one or combinations of whole, partially or fully skimmed, concentrated or powdered milk, buttermilk powder, concentrated or powdered whey, milk protein (such as whey proteins, whey protein concentrates, soluble milk proteins, edible casein and caseinates), cream, butter or milk fat—all of which have been manufactured from raw materials that have been at least pasteurized) by the action of specific microorganisms, which results in a reduction of the pH and coagulation.

2. Probiotics, prebiotics and synbiotics

Probiotics, prebiotics, and associated ingredients might add an attractive dimension to cultured dairy products for augmenting current demand for functional foods. Probiotic fermented milks, is one major segment amongst fermented milks that has tremendous potential for growth and development. Probiotics and cultured dairy products have an expanding volume of scientific data and a long list of claims on their beneficial health effects. Some of these health effects, such as relief of lactose mal-digestion symptoms and shortening of rotavirus diarrhea appear to be well documented and generally accepted. Immune modulation has also been well documented. For other health benefits such as treatment of irritable bowel syndrome, urinary tract infections, superficial bladder cancer, nutritional management of food allergies in infants, very promising results are available for selected probiotic strains. Prebiotics is another important aspect linking gut health and probiotics. At present, most searches for prebiotics are directed toward the growth of lactic acid-producing microorganisms. This is due to their purported health-promoting properties. However, it may be that future developments in the study of prebiotics may include aspects of their effect on pathogenic flora components. Another possibility in microflora management procedures is the use of synbiotics, in which probiotics and prebiotics are used in combination. The living microbial additions (probiotics) may be used in conjunction with specific substrates (prebiotics) for growth (e.g., an FOS in conjunction with a bifidobacterial strain or lactitol in conjunction with a lactobacillus organism). This combination could improve the survival of the probiotic organism, because its specific substrate is readily available for its fermentation, and result in advantages to the host that the live microorganism and prebiotic offer.

3. Fortification with physiologically active ingredients

Consumption of functional foods containing nutraceuticals is being highly encouraged, thus fermented milks produced with incorporation of these ingredients with specific health benefits could be of potential interest. Some of the functional ingredients designed to enhance consumer appeal, which may be incorporated into fermented milks, include:
3.1 Essential minerals and vitamins

Certain minerals like Calcium claimed to prevent osteoporosis, cancer and control hypertension can be fortified in cultured milks. An attempt to fortify yoghurt with calcium salts revealed that yoghurt is a suitable vehicle for fortification with calcium salts and calcium content of the fortified yoghurts could be increased with about 34.3, 37.6, and 39.4% by addition of Ca Lactate, Ca Gluconate and Ca Lactate + Ca Gluconate, respectively (Pirkul et al., 1997). Antioxidant vitamins (C and E) to prevent cancer, cardiovascular disease, and cataracts as well as multivitamin-mineral mixes are being incorporated in fat free cultured milks to provide meal replacements for consumers within a targeted niche (Chandan, 1999).

3.2 Dietary fibers

The beneficial role of dietary fibre in human nutrition has lead to a growing demand for incorporation of novel fibres into foods. There is little information about fiber fortification in cultured dairy products however various fibers like psyllium, guar gum, gum acacia, oat fiber, and soy components can be used. In one experiment, pectin and raspberry concentrate was incorporated in commercial stirred yogurt samples, increasing the consistency and it was found that yogurt with pectin was more shear stable in comparison with yogurt with raspberry concentrate (Ramaswamy and Basak 1992).

3.3 ω-3 fatty acids

Milk fat composition in dairy products can be altered by reducing the ratio of saturated to unsaturated fatty acids and increasing the contents of fatty acids that are more desirable for human nutrition, such as the ω-3 polyunsaturated fatty acids (PUFAs). The importance of ω-3 fatty acids like α-linolenic has been widely publicized because they are precursors of important long-chain fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which cannot be synthesized in the human body. Yet they are vital for the normal functioning and development of the brain, and are believed to reduce plaque formation in the arteries. They are also claimed to exert cancer inhibition, anti-allergy effects and improvement in learning ability.

3.4 Isoflavones

These are functional ingredients of a more recent interest, even though their commercial source, soy beans have been consumed for over 5000 yrs. Isoflavones are part of the di-phenol compounds, called “phytooestrogens,” which are structurally and functionally similar to estradiol, the human estrogen, but much less potent. Because of this similarity, isoflavones were suggested to have preventive effects for many kinds of hormone-dependent diseases.

3.5 Phytosterols and phytostanols

Oil-based products enriched with plant stanol esters can lower low-density lipoprotein (LDL) cholesterol concentrations by 10–14% and Mensink et al. (2002) concluded that low-fat yoghurt enriched with plant stanol esters lowers LDL cholesterol to the same extent as oil-based products within 1-week. Recently, Awaishshe et al. (2005) prepared yoghurts from modified milk base containing three important nutraceuticals, namely omega-3-fatty acids, isoflavones and phytosterols. The nutraceuticals appeared to have no adverse effect on flavour and storage trials at 5ºC showed that the viability of the probiotic cultures was retained over 15 days.
3.6 Gamma-aminobutyric acid (GABA)

It is an amino acid that has long been reported to lower blood pressure by intravenous administration in experimental animals and in human subjects. GABA is present in many vegetables and fruits but not in dairy products. However, the effect of dietary GABA has attracted little attention as a factor that may influence blood pressure. A novel fermented milk product containing GABA was reported to lower blood pressure in people with mild hypertension (Inoue et al., 2003).

4. Use of biothickeners -- EPS Cultures

At present isolated strains of Lactococcus species and thermophilic LAB are used extensively in the manufacture of fermented milks. They are of commercial interest as they act as biothickeners and aid in enhancing texture, mouthfeel and stability of the product. Kumar (2000) determined that dahi prepared using EPS culture had better body and texture and exhibited little syneresis. Folkenberg et al. (2005) produced set yoghurts with seven different exopolysaccharide producing starter cultures and observed that yoghurts in which the EPS were associated with protein had high ropiness, low serum separation and appeared more resistant to stirring.

5. Low calorie / Low fat fermented milks

With billion of dollars spent on dieting each year, consumers desire for nutritious low calorie dairy products continues to grow and consumption of low - or nonfat dairy products has increased in recognition of their health benefits, and consumers’ health problems. Various fat replacers and replacer blends that have been used to produce low fat/low calorie cultured dairy products and the technically developed fat substitutes are divided into 2 main types: modified starches or proteins which have good emulsifying or gel properties along with low calorie values; and modified fat/oil based products that contain bonds resistant to digestion, e.g., glycerol ethers and complex carbohydrates or fatty acid esters. Guven (2005) concluded that yogurt samples containing 1% of inulin showed similar characteristics to the control yogurt containing 3% of milk fat. However, increased use of inulin in fat-free yogurt negatively influenced some physical properties of yogurt, i.e. whey separation, consistency and organoleptic scores. Non-nutritive sweeteners can be used to impart an attractive calorie reduction in fermented milks. Ten different plain and fruit yoghurts were prepared using sucrose, Aspartame, sorbitol, calcium saccharine, sodium saccharine, fructose, Acesulfame-K, dihydrochalcone, sucrose plus monoammonium glycyrrhizinate (MAG) and fructose plus MAG as sweeteners wherein Aspartame yoghurt was reported to be the most preferred on the basis of consumer panel (Keating and White, 1990).

6. Fruits and fermented milks – product diversification

Recently there has been an increased trend to fortify cultured milk products with fruit juices/pulps. Fruits are rich sources of various important phyto- nutrients namely, vitamins, minerals, antioxidants and dietary fibers. Shukla et al. (1987) reviewed various methods of preparation of fruit yoghurt. Numerous other researchers have outlined processes for making fruit yoghurt with fruit concentration mainly ranging from 4-20%. Addition of mango pulp more then 4.0% was reported to adversely affect delicate yoghurt flavor and also the body and texture irrespective of homogenization pressure (Balasubramanyam and Kulkarni, 1991). Suitability of different fruits i.e. mango, sapota, papaya, pineapple, kokum @ 10, 15, 20% levels each was studied for preparation of fruit yoghurt and it was concluded that that mango pulp and pineapple juice could be used satisfactorily up to 20% level. However, sapota pulp, papaya pulp and kokum juice...
produced inferior quality yoghurt (Desai et al., 1991).

7. Whey based fermented milks

Several studies have focused on the use of milk whey in yoghurt making and use of whey powder or whey–milk powder mixtures. This leads to the increase of milk total solid content in order to provide better consistency, texture and creaminess to the product. According to Penna (1997) lactic beverages are a series of products including those prepared with milk and whey. Industrial lactic fermented beverages are formulated products containing yogurt, whey, fruit juice or pulp, flavor, other raw materials and allowed additives. However, yogurt microorganisms should be plenty and alive in the final product. Good quality whey based fermented milk drink containing 2.5% fat and 10% sugar was prepared by Otero et al. (1995). Macedo et al. (1999) prepared low cost, probiotic whey milk beverage using buffalo milk cheese whey, cow skim milk and soymilk. Lassi like cultured milk-whey beverages have been developed using paneer whey (Mittal, 2003) and cheese whey (Kumar, 2004).

8. Conclusion

It is evident that the market for fermented milks is booming specially probiotics and those with special added ingredients. Modern consumers are increasingly interested in their personal health, and expect the food that they eat to be healthy or even capable of preventing illness. Producers and marketers of cultured milks are making every effort to keep them growing through product development and packaging innovations while delivering a ‘good for you’ flavorful products suited for all occasions of gastronomic indulgence. A major consideration in the continued development and success of ever growing fermented milk market is communication. This is linked to other important factors such as development of supporting scientific documentation; a health claims strategy and successful presentation.

9. Selected Reading


1. Introduction

Ice cream is a frozen dairy product prepared by suitable blending and processing of cream/butter and other dairy products together with sugar and flavour, with or without stabilizers or colour, and with the incorporation of air during the freezing process.

2. Indian Ice cream Industry

India has the right climate for ice cream consumption but has a very low per capita consumption, which is approximately 300 ml per annum as against the world average of 2.3 litres per annum, which means a large untapped potential business awaits to be explored. Seeing this enormous business opportunity, the war has already begun to grab most of this pie among the national players by increasing their production and international players trying to find ways to enter the Indian markets.

Gujarat and Delhi together account for 30 per cent of the country’s Rs 3,000-crore ice cream market that is expected to double to the value of Rs 6,000 crore by 2014-15. Almost 35 per cent of the ice creams sold in the country are consumed in the western region with Ahmedabad being the main market, followed by 30 per cent in the north, 20 per cent in the south and 15 per cent in the eastern and central India, says a study on ice-cream industry conducted by the Associated Chambers of Commerce and Industry of India (ASSOCHAM). To maintain the growth momentum, it is necessary to Innovate & introduce newer trends in the market so that the customer is provided with options to satisfy his taste buds.

3. Newer trends in ice cream industry

Even though ice cream manufacturers are looking to inculcate the habit of consuming ice cream as a dessert in Indian households, impulse products such as sticks, cones and cups have much higher penetration through refrigerated push-carts and bicycles in the country. The higher volume packs comprising take-home ice cream also present lower margins and take up more storage space, which is at a premium due to the limited capacity possessed by the individual retail channels mentioned above. Also ice cream manufacturers have cut throat competition in order to gain the market share. The only other alternative for the ice cream industry is to look for newer trends in order to penetrate newer market, increase the consumption pattern and to get better margins to sustain in the highly competitive market.

There are many trends which are being followed in different parts of the world. Some of the trends get extended from different food segment which include fortification, low calorie, all natural etc. But when we discuss about the trend, it should cover a broad range of spectrum so that most of the customer wants are covered. These trends can be broadly categorized in to three main “Mega Trends” which include:

i. Health & Well-being: Physical & mental well-being
ii. Pleasure / Fun: Sensorial, emotional & out of the world experience
iii. Convenience & Quality: The time factors like easy to carry, convenient format etc.

3.1 Health & Well-being

Currently, this is one of the major consumer trends in the food industry. Consumers continue to seek multiple ways to enhance their health to prevent diseases, and to promote healthy aging by paying more attention to what they are eating and how it benefits their health. Today consumers are more concerned about their weight, cardio-health, digestive-health and immunity than ever before. Since health is a major concern of consumers, manufacturers are finding new ways to incorporate natural and innovative ingredients into food products for health benefits. While there is a lot of information out there what we should and shouldn’t eat, few of these trends top the list for healthy eating.

- Fruits and vegetables moving up the agenda
- People are looking for low calorie products
- Natural & beauty finding preference with female customers
- Product with functional ingredients

Ice cream is considered as a high calorie product and hence finds fewer takers in health conscious and obese customers. Targeting the customer base with a well defined product which may have all natural product, low calorie formulation, product with higher amounts of natural fruits, enriched products with newer functional ingredients, vitamins & minerals which meet their requirement.

3.2 Pleasure / Fun

Consumers are also looking at the exotic foods, drinks and miracle fruits to get pleasure from them, as they taste different and also provide great health benefits. Exotic or super fruits were a dream in the past but are now a reality as they can easily be found in a local market. Working couples who are short on time and young adults are seeking energy, power and performance from these foods. Thus it becomes very important for the ice cream developer to incorporate these attributes in the product.

3.3 Convenience & Quality

Despite the economic tough times, consumers still want a convenient, quick, healthy, tasty and sophisticated food experience and want it at a lower price. Consumer demands include ease in purchasing, convenient in handling, good nutritional value, flavor and food value. They want product to at affordable price but do not want to compromise on quality. Customer wants product which can be purchased on-the-go, packed in attractive & easy to handle format, good taste and nutrition.

4. Innovative Ice cream & Frozen Desserts

Understanding the “Mega Trends” is important and it acts as a tool for food developer to create product for specific customers. In India, customers are very specific; a unique concept will only work if two attributes are met. First, it should be affordable and second, the taste of the product should match customers taste buds. Some of the innovative products and their formulation keeping in view the trends, affordability and taste are discussed below.

4.1 Sugar free ice cream

Low-sugar has become a huge trend in the food industry. Food makers are rushing to meet demand from consumers concerned with their waistlines and healthier eating by providing an array of new products. India being the diabetic capital of the world, the
A sugar free product is becoming more & more popular. This term was relatively unheard of just a few years ago, but low-glycemic foods are establishing themselves as a viable niche in the mainstream.

Ice cream is one of the important dairy products which is liked by people of all age groups. It provides energy, nutrients and is a good source of calcium. Diabetic people stay away from ice cream due to higher amount of sugar used in the manufacture of ice cream. In this context it is important for the companies to develop the sugar-free ice cream with ingredients having very low glycemic index to cater the needs of diabetic population without affecting their blood glucose level. The formulation can be made with low fat content so that the product is recommended for health conscious people.

<table>
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<td>SMP</td>
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<tr>
<td>Cream</td>
<td>30.00</td>
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<tr>
<td>Bulking Agents</td>
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<tr>
<td>Stabilizer &amp; emulsifier system</td>
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<tr>
<td>Non-calorific Sweeteners</td>
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<tr>
<td>Colour &amp; Flavour</td>
<td>As per taste</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

4.2 Frozen Yoghurt

Frozen yogurt is a frozen dessert containing yogurt or other milk products. In India it will fall under proprietary product category. It is slightly more acidic than ice cream. Frozen yogurt usually consists of milk solids, sweetener, milk fat, yogurt culture, coloring, and flavoring substances.

4.2.1 Benefits/Differentiators

- Frozen yoghurt will be having less fat (0.5 to 5 %) compared to regular ice cream (up to 10 %), thus suitable for calorie conscious people.
- It contains live organisms which help in digestion & immunity (depending on the microbial strains used)
- Due to presence of live organisms the lactose present in the milk is converted to lactic acid, thus can be suitable for lactose intolerance people

Formulation of frozen yoghurt mainly consist of plain mix & yoghurt mix in the ratio of 50:50 or 70:30 depending upon the taste (tartness in the product required), over run ranging from 50-100 % and hardened before consuming. Today in India, many frozen yoghurt parlors have come-up serving soft frozen yoghurt and creating awareness among the customers.

4.3. Sorbet

Sorbet is a category of frozen dessert products which are made up of water, sugar, fruit & functional systems. Sorbets can be defined as water ice with a certain overrun, typically 20-100%. Most sorbets are fruit flavour types, but also more unusual flavour types and combinations are seen today.
### Sorbet formulation

<table>
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<th>Ingredients</th>
<th>Percent</th>
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<td>Sugar</td>
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<tr>
<td>Stabilizer &amp; Emulsifier System</td>
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<td>Water, Citric acid incl.</td>
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<tr>
<td><strong>Total</strong></td>
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</tr>
</tbody>
</table>

4.4 Gelato

Gelato is also a frozen dessert and contains the same ingredients as Ice Cream viz., milk, cream, sugar and stabilizer/emulsifier system. Most of the Gelato is made up of natural ingredients with no synthetic flavors or essences. Gelato has a lower fat content of approx. 4 per cent as compared to regular ice cream which is higher at 10 percent or more. Over run of only 35 per cent is achieved to make it more compact, silky consistency as compared to industrial ice cream which has an 80-100 per cent over run.

5. Innovative Inclusions for ice cream

Traditionally ice cream in India has dry fruits and nuts like almonds, cashew, walnut, pista, kishmish etc. as inclusions. Today, there are over 100 newer inclusions available in the market. But choosing them considering the taste buds of customers is very important. Some of the newer inclusions which are now used /considered for use in ice creams and frozen desserts include:

- Exotic fruit preparations with fruit pieces (like blueberry, cranberry, lemon etc.)
- Sweetened coconut dices / shreds giving real feel of coconut
- Cookies /cakes / biscuits
- Extruded wheat / rice crispies with chocolate coating
- Multi - colored chocolate pieces with different fruit flavours
- Exotic nuts paste (hazelnut, pista, walnut etc.) to give natural feel & taste to the product
- Yoghurt flavored chocolate pieces etc.

6. Innovative Technologies in Ice cream Industry

6.1 Extrusion Technology

Ice cream in different shapes, sizes will fancy the customers particularly young adults & children. The demand for these extruded products has seen an exponential curve over the last few years. Arrival of premium ice cream manufacturers like Haagan Dazs, Movenpick, London Dairy etc. have stirred a great level of competition in extruded products category. The product should be frozen to certain over run so that it retains its shape after extrusion until it enters the hardening tunnel which is maintained at – 35 ºC.

The main component of the machine is the extruder which can be of your choice of shape & size. It can be slices, ball cone, ice cream logs etc. The ice cream from continuous freezer is extruded through the mould which forms the required shape and size. By placing different extrusion devices inside the mould, faces with eyes, mouth and other
designs can be formed. Once the design is formed, a heated single-wire cutter produces slices which are accurately positioned on the conveyor plates. If the product is stick product, stick insertion happens before the wire cutting is done. The conveyor passes into the hardening tunnel for 15-30 mins depending on the machine speed. The product which is coming out of the tunnel is ready to eat product. For stick products, there are pincers which lift these products by picking the stick and chocolate enrobing is accomplished prior to final packaging. A metallic hammer is used to ensure the release of product from the conveyor plate.

6.2 Deep Blue Technology

Deep Blue is the brand name of Tetra Hoyer for their low temperature ice cream concept. At the heart of this technology is the second stage freezer, which cools low temperature ice cream to –12 to –15 °C (10 to 5 °F) where it becomes very viscous, rather like soft clay. It is ideal for shaping into a variety of new products.

Low temperature ice cream undergoes a cold kneading process in this freezer, creating a finely structured ice cream with a smoother feel and creamier taste. You can now achieve excellent creaminess even with low-fat ice cream so that healthier, low fat products can taste just as good as any traditional ice cream. This helps in reducing raw material costs either by reducing the amount of expensive ingredients such as cream or butterfat or by using less expensive ingredients like vegetable fat.

7. Way Forward

Traditional factors such as consumer demand for indulgence, growth in newer formats and pricing pressures on raw materials such as sugar and dairy continue to influence the ice cream market. However, some important emergent trends will drive consumption in new directions. Health is the dominant theme affecting new product development and innovation in the ice cream industry. Hence the ice cream product developer should look at developing low calorie formulations, sugar free solutions, inclusions of more & exotic fruits and reducing the use of artificial ingredients to give healthier options to the new generation customers.

8. Selected reading

Dairy Processing Handbook

www.assocham.org

www.tetrapak.com

www.mintel.com
1. Introduction

Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers. In this regard, functional foods play an outstanding role. So the current trend is functional foods development, to enhance the health attributes of widely consumed foods by fortifying with functional food ingredients. Commonly used functional ingredients are dietary fiber, phytosterol, ω-3 fatty acid, probiotics, herbs, etc. Global functional foods market is growing annually at 7.4%, whereas in India it is growing @ 12-15% (Patel, 2011).

Cardiovascular diseases (CVDs) are the foremost cause of death in the world as well as in India, accounting for 25-30% of all deaths as per the World Report of the WHO. In India in the past five decade, rate of coronary heart disease among urban populations has risen from 4% to 11%. It is estimated that almost 60% of world’s cardiac patients is in India (WHO, 2010). So the most common used functional ingredients are to reduce serum cholesterol levels for cardiovascular health. Among these functional ingredients, inulin, phytosterol and ω-3 fatty acids have important role.

Butter, the traditional spread for bread is now avoided due to poor spreadability, high saturated fat and cholesterol content. The annual growth rate of cheese production in India is 10-15% and 90% cheese is consumed as processed cheese and processed cheese spread. Processed cheese spread contains a lower amount of fat and higher amount of protein compared to any low fat table spread. It contains not only that protein and fat are in pre-digested form, also it contains calcium, phosphorus, riboflavin and other vitamins in a concentrated form and contains health beneficial bioactive peptides. In this direction, cheese spread can provide nutritionally superior spread for bread and it can be incorporated with functional ingredients.

2. Functional ingredients

2.1 Inulin

Dietary fibers are associated with lipid metabolism, has shown to have hypercholesterolemic properties. Inulin is a soluble dietary fiber. It is present in many regularly consumed vegetables, fruits and cereals, including leek, onion, garlic, wheat, chicory, artichoke, banana etc. Inulin supports a healthy circulatory (blood), increases mineral absorption in the colon, prevents hypoglycemia, curbs appetite, eliminates constipation etc.

Pagliarini and Beatrice (1994) prepared low fat Mozzarella cheese by adding inulin which significantly improved the sensory quality particularly increasing softness and wateriness which were perceived as positive sensory attributes by consumers. El-Nagar and El-Aty (2004) manufactured Karish cheese from reconstituted skim milk (11% SNF) by using fat replacer inulin at the rate of 0.5, 1.0 and 1.5%. Due to inulin addition hardness and elasticity decreased but adhesiveness, cohesiveness, gumminess and chewiness increased. Hennelly et al. (2006) compared the use of shear-induced inulin gels and heated inulin
solutions to replace 63% of the fat in imitation cheese. The melting behaviour and the variation of the elasticity modulus with temperature and the cheese microstructure were not affected by the form in which inulin was added (inulin gel or inulin solution). They also observed that at equivalent moisture levels, the inulin cheeses had significantly higher hardness values than the control sample with fat; however, there was no difference in hardness between the cheeses containing different levels of inulin (5% or 13.75%).

Koca and Metin (2004) studied the possibility to obtain low-fat fresh Kashar cheese with a 70% fat reduction using long-chain inulin. Low-fat control cheese due to its high protein content was significantly harder, more elastic, gummier and chewier than the full-fat control cheese. The authors comment that it was not surprising because fat breaks the protein matrix and acts as lubricant to provide a softer texture. Addition of 5% inulin to the low-fat cheese resulted in a significantly lower hardness compared to low-fat control cheese but slightly higher than that of full-fat control cheese. Functional Quarg cheese was developed by Gahane (2008) in which inulin was used @ 8-12% of curd and oat fiber and soy fiber @1-3% of the curd in order to provide sufficient concentration of dietary fiber in the product. The study revealed addition of inulin, oat fiber, soy fiber up to 10, 1 and 1% resulted in an increase in all the sensory scores of all attributes studied and also found very close to control.

2.2 Phytosterol

Phytosterol is waxy substance. It differs from cholesterol by the presence of an extra methyl or ethyl group on the cholesterol side chain at the C-24 position. Phytostanol is saturated phytosterols, that is, they have no double bonds in the sterol ring. Phytosterol founds in significant amounts in cereal grains, seeds, nuts, fruits and vegetables. It reduces intestinal absorption of both dietary and endogenously produced cholesterol without any decrease in the levels of HDL-cholesterol or triglycerides. This interference with absorption is probably related to the similarity in the chemical structures of phytosterols, stanols and cholesterol (Kanawjia and Makhal, 2008).

Kwak et al. (2005) studied the effect of phytosterol ester addition on lowering blood cholesterol in cholesterol-reduced (91%) Cheddar cheese. By addition of phytosterol all rheological properties increased during ripening period, rancid, bitterness and off-flavor intensities increased significantly, while texture was decreased during ripening. Total blood cholesterol was reduced by 18% when rats were fed Cheddar cheese, treated with 8% phytosterol. Gahane (2008), reported that in fiber enriched Quarg cheese different levels (2, 3 and 5%) of phytosterol ester addition up to 5% there was no significant change in sensory quality.

2.3 ω-3 Fatty Acids

They consist of the following: α-linolenic acid (ALA), stearidonic acid, docosapentaenoic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). ω-3 fatty acids come from two main sources: marine (fish oils) and plant (flax and canola oil). These ω-3 fatty acids have important role against coronary heart disease (CHD), thrombosis, atherosclerosis, inflammation, cancer, autoimmune disorders, aging, cardiovascular disease, rheumatoid arthritis, Parkinson's disease, Alzheimer's disease and helps for development of brain. Enrichment of foods with this ω-3 fatty acid is problematic due to the high susceptibility of the highly unsaturated fatty acids to oxidative deterioration and rancidity but this defect is being rectified and lot of research is going on to improve the sensory and oxidative stability of ω-3 fortified foods.
Martini et al. (2009) fortified 50% reduced fat Cheddar cheese with DHA and EPA to obtain 3 final fortification levels [18, 35 and 71 mg of DHA/EPA per serving (28 g) of cheese] representing 10, 20, and 40% of the suggested daily intake level for DHA/EPA. No differences were found in the oxidized and rancid flavors as a consequence of DHA/EPA fortification, with only slight intensities of these flavors. Cheese with low fortification levels (18 and 35 mg of DHA/EPA per serving size) did not develop significant fishy off-flavor compared with the control, whereas at the highest fortification level (71 mg of DHA/EPA per serving size) the fishy off-flavor was significantly stronger in young cheeses. The fishy flavor decreased as a function of age and became nonsignificant compared with the control at 3 months of storage. Even though fishy flavors were detected in the fortified cheeses, the DHA/EPA content during storage remained constant and complied with the suggested values for food fortification. Results obtained from this research indicate that 50% reduced-fat Cheddar cheese aged for 3 months can be used as a vehicle for delivery of ω-3 fatty acids without generation of off-flavors. Kolanowski and Weißbrodt (2007) reported that fish oil can be incorporated to reduced fat soft cheese, full fat soft cheese, spreadable fresh cheese (Philadelphia type, not flavoured), garlic flavoured spreadable fresh cheese (Philadelphia type), processed cheese (not flavoured) and garlic flavoured processed cheese upto 0.3, 0.5, 1.5, 2.0, 4.0 and 6.0% respectively without affecting sensory quality of the products. Such dairy products maintained a constant sensory quality during 4 weeks of storage.

3. Selected Reading
Receiving Cheddar cheese

Selection for blending
(1½ month old-75% + 6 month old-25%)

Tempering (18-20°C/24 h) & cleaning

Quartering & grinding

Analyzing
[moisture, fat, salt, titratable acidity & pH]

Addition of calculated amount of water, salt & emulsifier to maintain final composition of controlled processed cheese spread:
moisture – 60%, salt – 2% & tri-sodium citrate – 2.5%

Heating (85-90°C/3-5 min) & stirring

Addition of inulin, phytosterol & ω-3 fatty acids & mixing

Hot packaging

**Fig. 1:** Process flow diagram for manufacture of functional processed cheese spread
Innovations in Ready-to-Drink (RTD) Non-Alcoholic Beverages

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1. Introduction

India has witnessed radical shift in consumption of non-alcoholic drinks over the recent past. Increasing middle class population, rapid urbanisation and rising disposable income are some of the major factors fuelling this growth. Besides this, growing health consciousness among India’s young population has brought about a revolution in the Indian non-alcoholic drinks market. It has been seen that cola and other carbonated drinks sales have fallen dramatically due to rising health concerns and this seems to have benefited the country’s non-carbonated drinks market such as fruit juices, fruit drinks and dairy drinks. A report from the Associated Chambers of Commerce and Industry (ASSOCHAM) in India reveals that current market of non-alcoholic beverages in India is US$ 1.2 billion and it would grow to US$ 2.3 billion by 2015 at the annual growth rate of 20%. The current consumption of non-alcoholic beverages stands at 175 million litres and estimated to touch 35 billion litres by 2015. It further revealed that carbonated drinks captured 30% share among non-alcoholic beverages and accounts for US$ 370 million, fruit drink segment accounts for US$ 250 million and energy drink market accounts for US$ 100 million in terms of value.

![Figure 1: Classification of beverages](image-url)
2. Classification of beverages

2.1 Dairy beverages

Milk provides more satiating nutrients than other beverages. Three cups of milk are recommended daily by American Dietary Guidelines released in 2005. The interest in developing dairy beverages is rising day-by-day, driven by the market potential for the health and well-being of the consumers. Fermented dairy beverages make up an important contribution to the human diet in many countries because fermentation is an inexpensive technology, which preserves the food, improves its nutritional value and enhances its sensory properties.

2.1.1 Flavoured milk

Flavored milk is popular in traditional flavors such as kesar, elaichi and chocolate, recently innovative flavors including butterscotch, strawberry, vanilla, and thandai are enter into the market. Flavoured milk is the most attractive choice for children. It has been considered as highly palatable and nourishing beverage. Processing and packaging technology adapted for flavoured milk widely in India is filling flavoured milk into glass bottles, capping and followed by retort sterilization. Recently major dairy giants launched the flavoured milk in sterilizable plastic bottles and few producers launched DHA and EPA fortified flavoured milk in India. Sterilization was shown to induce lipid oxidation in dairy beverages but at the same time it produces Maillard Reaction Products (MRPs) that provide antioxidant properties (Giroux et al., 2008). Recently, it has been shown that pre-heated milk protein-sugar blends provide MRPs in the early stage of sterilization, which efficiently prevents lipid oxidation of dairy beverages (Giroux et al., 2010).

![Figure 2: Direct Liquid Inoculation System](image)

Source: Tetra Pak

2.1.2 Probiotic dairy beverages

The development of probiotics in the last two decades has signalled an important advance in the food industry. The probiotic microorganisms also have been directly incorporated into the drinks after the heat treatment. The key to the development of this second generation probiotic products is the design of special Direct Liquid Inoculation System (Fig. 2) by Tetra Pak commercially available as ‘Tetra FlexDos’. It allows food producers to add the probiotic bacteria directly to the beverages after heat treatment and just before
they are filled into the cartons. The innovation is expected to significantly boost the market for the probiotic beverages, which have so far been restricted by the delicate nature of the ingredient and concerns over the contamination.

2.1.3 Yoghurt drinks

Yogurt is a conventional food known for its therapeutic, nutritional, and sensory properties. One possible method of enhancing those properties further is by creating yogurt drinks. Hydrocolloids have been widely used in texture stabilization of fermented milk products. High methoxyl pectin is preferred in acidic milk beverages as a stabilizer. Pectin molecules interact with casein through calcium ions and prevent their aggregation, sedimentation and hence serum separation by ionic and steric stabilization in acidic milk beverages (Lucey et al., 1999). Yogurt drinks are highly desired by consumers due to their healthy, convenient, and portable characteristics. They are being marketed and modified successfully to fit the target populations, separate formulations for kids and adults are in the market with varying sugar and fat level. Yogurt drinks, mainly the low-fat and non-fat varieties (326 KJ/serving), are increasingly popular among the adult female population. Adding an indigestible carbohydrate and a prebiotic like inulin to yogurt drinks, which has been linked to improved colon health, and increased absorption of calcium and minerals increases the marketability of such products. High protein yoghurt drink containing higher α-lactalbumin suppressed hunger significantly in comparison to control (Hursel et al., 2010). In a study by Gonzalez et al., (2011) reveals that the whole milk based yogurt beverages were liked over the skim milk yogurt drink. The whole milk beverage containing the prebiotic (fructooligosaccharide) being the most acceptable. However, symbiotic yoghurt drink (fructooligosaccharide and Lactobacillus acidophilus) was not preferred due to higher intensities of sour and yeasty aroma.

2.2 Soy beverages

According to Beverage Marketing Corporation (2010), soymilk has been one of the fastest growing categories in the general beverage marketplace and secured a much higher growth rate than the dairy milk segment over the last decade. Growth in soymilk has been attributed to improved health-related claims and consumer perceptions. Flurry of soy milk brands, appealing and convenient packaging and bunch of various flavors available such as chocolate, vanilla, strawberries etc escalates soy milk competency over flavoured diary milk. Soy milk contains low fat, carbohydrate, calcium, phosphorus, and riboflavin, but higher amount of protein, iron, thiamine, and niacin in comparison with cows’ milk. Soy milk is characterized by beany flavor which can be masked by introducing suitable flavours. Currently, calcium fortified soy milk are available as alternative to dairy beverages and compete with white and flavored milk. These beverages are much useful for lactose intolerance population.

2.3 Fruit beverages

2.3.1 Fruit Juice

Fruit juices are very popular due to their perceived naturalness and as a vector of micronutrients. The health trend continues to dominate innovation in the fruit juice market. Juices provide a convenient and tasty option. The most innovative region in juices is Europe. The Asia-Pacific region has the second highest share of juice launches, with a 33.2% share in 2008 (www.globalbusinessinsights.com). The ‘Wellness’ category is the one of the fastest growing retail categories in the juice market. Obesity and health concerns are becoming issues for all western countries, and all companies in the Wellness category are seeing massive growth. The most common product tag on juices since 2005
has been ‘high vitamins’. This is a result of manufacturers choosing to use fruits in products that contain high antioxidants in order to capitalize on greater consumer understanding of the role they play in boosting general health. The ‘high in antioxidants’ tag was featured on 0.8% of juices launched in 2005 and has grown to 2.8% in 2008. Exotic fruit juice with high vitamin content is also a new trend emerged in western world with the launch of acerola juice (acerola fruit is native to the Caribbean Islands) which has 25 times the vitamin content of oranges.

The ACE drinks are very much popular in Europe and USA wherein the natural functionality of antioxidants is used in form of pro vitamin A. Antioxidants are found in high quantities in certain fruits (e.g. in superfruits such as pomegranate, cranberry or acerola) as well as in some vegetable varieties, and they help prevent damage to the body from free radicals. Cholesterol lowering juice has been introduced into the market by incorporating a limonin glucoside (oxygenated triterpenoid compound) present naturally in citrus fruits and can be extracted as a by-product in citrus processing plants (nutraingredients-USA.com). Dalgard (2009) reported that orange juice and blackcurrant juice are better than vitamin E supplement in reducing the levels of C-reactive protein (CPR), a protein produced in the liver and is known marker for inflammation. An increased level of CRP has been found to be a good predictor for the onset of both type-2 diabetes and cardiovascular disease.

Several non-thermal processing technologies have been evaluated for fruit juice processing, among them potential technologies which have scope for commercialization are High Hydrostatic Pressure (HHP) processing, Pulsed Electric Field (PEF) processing and Ultraviolet (UV) light irradiation. These technologies are helpful to preserve the freshness in fruit juice and minimal effect on heat sensitive micronutrients such as vitamins and other bioactive components.

2.3.2 Smoothies

Smoothies are blended drinks consisting of a number of ingredients including fruit (or less commonly vegetables), fruit juice, ice, yoghurt and milk. There are three main types of smoothies 1) fruit only 2) fruit and dairy 3) functional. Functional smoothies, such as those that contain probiotics, have appeared only very recently on the market. Smoothies are commonly sold as a drink, snack or meal alternative and are available either ready-made or made-to-order. They provide a convenient way of consuming fruit and dairy products where yoghurt and milk are included.

2.3.3 Milk and fruit drinks

Milk and fruit drinks have established themselves well in the European market and have grown in recent years by around 7%. New market opportunities are arising in Europe and China. The motivation for using dairy and fruit drinks is not just refreshment, but also issues such as healthy nutrition, functional ingredients, satisfaction and enjoyment. It is being the common practice to have milk shakes prepared freshly by blending milk and fruit pulps like mango, grape etc in India. Now, technological advances are being made to process these drinks commercially, and to extend their shelf life. Recently, Coca Cola launched ‘Maaza Milky Delite’ prepared with mango pulp and milk solids in India (ET Bureau Aug. 18, 2010).

2.4 Sports beverages

The production and sale of sports drinks is lucrative as demand is immense. U.S. market alone is estimated at being worth over $1.5 billion a year. Sports drinks are becoming increasingly popular as we are all being encouraged to adopt a healthier lifestyle with
regular exercise. Sports drinks are typically formulated to: (1) prevent dehydration, (2) supply carbohydrates to augment available energy, (3) provide electrolytes to replace losses due to perspiration, and (4) be highly palatable. The beverages usually contain between 6 and 8% carbohydrate with slight variation in the combination of carbohydrate sources used by manufacturers (glucose, fructose, sucrose, maltodextrins, high fructose corn syrup etc). Small amounts of electrolytes, generally sodium, potassium, and chloride, are added to sports drinks to improve palatability and to maintain electrolyte balance. The addition of electrolytes affects beverage osmolality which in turn influences the rate of absorption of the fluid and its contents. The pH of the sports drinks vary from 2.4 to 4.5. Gatorade, popular sports drink, has pH ranges from 2.9 to 3.2 depending on flavour, and contains 20 mmol/L of sodium, 3.2 mmol/L of potassium, negligible calcium, and 13.9 mmol/L of citrate (Goodman et al., 2008). Not many innovations occurred in recent time except flavour modification.

2.5 Functional beverages

2.5.1 Energy drinks

The Indian energy drinks market is estimated at Rs. 500 crores and is expected to grow at a compounded annual growth rate of 25 % (Business Standard, 22 Oct. 2012). The energy drink market in India is dominated by Red Bull, Cloud 9 and Power Horse. Key constituents include caffeine, glucuronolactone, and taurine amongst other potentially performance enhancing ingredients. It also contains glucose and B group vitamins. Caffeine in energy drinks will provide the consumer the desirable effects of increased alertness, improved memory, and enhanced mood. Acute caffeine ingestion of 2–9 mg/kg body weight during aerobic exercise increases endurance and reduces fatigue. However, caffeine can have harmful physical consequences. Therefore, recently Food Safety & Standards Authority of India (FSSAI) has stated drinks containing a high level of caffeine should be categorised as ‘caffeinated drinks’. Currently, up to 145 ppm of caffeine is allowed in soft drinks and aerated sugar water and drinks containing more than this level could be called as ‘caffeinated drinks’ according to FSSAI. Taurine is known to modulate mood as well as stress and behavioural response. The mechanism of action for taurine within the central nervous system may involve a variety of neurotransmitter systems (Ramanathan et al., 1997). Degree of carbonation and flavour varies to suit regional preferences. Recently a study by Wortley et al., 2010, reported that energy drink consumption acutely increases platelet aggregation and decreases endothelial function thus it may lead to myocardial infarction.

3. Conclusion

Recent consumer trends such as convenience, health and wellness, value for money and indulgence created huge potential in non-alcoholic beverage market. Manufacture wish to introduce products in each category to tap the business potential with their brand value. It helps market players in product differentiation. It motivates researchers to diverge the innovation path to keep the momentum in market.

4. Suggested Reading

Beverage Marketing Corporatio. (2010). Soy Beverages in the U.S.


Cereal Based Fermented Dairy Beverages

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1. Introduction

Cereal crops are energy dense, containing 10000-15000 kJ/Kg, about 10-20 times more energy than most succulent fruits and vegetables. Nutritionally, they are important sources of dietary proteins, carbohydrates, the B complex of vitamins, vitamin E, iron, trace minerals and fibre. Cereal grains constitute a major source of dietary nutrients all over the world. Addition of cereals into milk not only enriches its mineral value but also supplements fibre. Fermentation further enhances the nutritive value, palatability and functionality of cereals by reducing the anti-nutritional factors. Development of technologies for the manufacture of cereal based fermented milk beverages will lead to better utilization of underutilized cereals like sorghum, pearl millet, finger millet etc.

The sensory or organoleptic characteristics of natural or processed foods are important for their acceptability and utilization. The animal foods such as milk, egg, fish, meat and their processed products are liked by people due to their desirable appearance, flavour, aroma and texture. Coarse grains like corn, sorghum, millet, barley etc. contain appreciable amounts of crude fibre and lack gluten like properties of wheat. The traditional foods prepared from these grains are often coarse in texture and lack flavour and aroma. Besides sensory characteristics, some of the cereals lack some essential amino acids and nutrients and contain anti-nutritional factors as well. Several methods have been employed to improve the nutritional quality of cereals. These include genetic improvement, amino acid fortification, and supplementation and complementation with protein concentrates or isolates or other protein rich sources such as grain legumes or defatted oilseed meals. The processing technologies employed include cooking, sprouting, milling and fermentation. Fermentation with natural microflora or pure cultures has been found to be most effective and highly beneficial for improving the nutritional quality of cereal based foods.

2. Traditional fermented milk cereal beverages

2.1 Pearl Millet and Sorghum Lassi (Raabdi)

*Raabdi* is a traditional popular beverage of Haryana, Rajasthan and Punjab. It is prepared by mixing coarse cereals/ cereal flour with buttermilk and then cooking the mixture. It can be served either hot or cold. The technology of producing these traditional fermented foods from cereals and milk solids remains a household art. Traditional process of *raabdi* preparation yields a product with limited shelf life (one to two days) with unpredictable sensory quality. Thus, several attempts were made to improve nutritional value, sensory characteristics and shelf-life of this traditional milk cereal beverage.

A technology has been developed for manufacturing pearl millet based and sorghum based fermented milk beverage. These products are similar to traditional *raabdi* and were named as *bajra lassi* and *jowar lassi*.

Milk solids and cereal flour were used for manufacturing these products. Further, both the milk solids and cereal solids were fermented together to increase the nutritive value of the final product. Development of these products was based on the selection of milk solids source; selection of level, form & stage of addition of cereal solids; fermentation
conditions and stabilization of developed product in terms of preventing sedimentation and wheying-off in the product during storage. Although traditional product is prepared from sour buttermilk, yet keeping in view the suitability for industrial production and easy availability, skim milk or standardized milk was selected as source of milk solids for development of this beverage. Cereal (Sorghum and pearl millet) solids were added to milk in three different forms viz. a) raw flour obtained from milling cereal grains, b) slurry obtained by grinding of soaked cereal grains and c) flour obtained after grinding of 24 h & 48 h germinated and dried cereal grains (malt). These solids were incorporated at two stages i.e. before fermentation and after fermentation. NCDC-167 starter culture is added for fermentation to obtain desirable acidity in the curd. After fermentation, the curd was broken and mixed with water, salt and spices to make the beverage. The beverage was then packaged in suitable size packages (LDPE pouches). The detailed flow diagram for preparation of raabdi like milk-cereal fermented beverage is given in figure 1 (Modha and Pal, 2011).

The shelf life of the product was found to be 7 days at refrigerated storage. So, attempts were also made to increase the shelf life of the product. For this, preservatives were used and in another study, this beverage was prepared in dried form which can be reconstituted at the time of consumption.

Fig. 1: Flow diagram for manufacturing bajra/ sorghum lassi
2.1.1 Pearl Millet and sorghum lassi in Ready-to-reconstitute form

There is a great demand of ready-to-reconstitute (RTR) products including dairy products in India. Efforts were therefore made to develop a technology for production of ready-to-reconstitute sorghum/pearl millet based fermented milk beverage by adopting spray drying method.

Milk solids were used in form of concentrated skim milk and cream (CSM), sorghum and pearl millet solids in form of malt. Concentrated standardized milk with different TS levels and pearl millet flour levels were tried.

Two cultures viz. NCDC-167 and NCDC-263 were used for fermentation of mixture having CSM and cereals malt. The optimum levels were decided on the basis of convenience in spray drying, and sensory evaluation and physicochemical properties of the reconstituted beverage. The effect of stage of addition of pearl millet and sorghum solids to milk solids was also studied. For this purpose two stages were followed viz. before fermentation and after fermentation. In the first case cereal malt was added to milk solids before inoculation of culture followed by fermentation. In the second case, the culture was added to CSM and fermentation was carried out, then the flour was added to the set curd. The product obtained by the addition of flour to CSM before fermentation stage was found to be more acceptable.

The mix having optimum levels of milk solids and cereal malt was heated to 90°C for 10 min, then after cooling to 37°C, it was inoculated with starter culture followed by incubation at the same temperature for 10-12 h. The fermented concentrated Raabdi-mix so obtained was blended with salt followed by passing through Fryma grinder to make smooth mass suitable for spray drying. Then it was spray dried at an inlet air temperature of 178 ± 2°C and an outlet temperature of 77 ± 2°C. The powder obtained was dry blended with spices and pectin. The detailed standardized method for manufacturing RTR sorghum/pearl millet based fermented milk beverage is given in figure 2. RTR products were analysed for gross composition and physico-chemical properties. The conditions of reconstitution of powder into beverage were also standardized.

2.1.2 Shelf life extension

Sorghum and pearl millet based beverages had a shelf life of about seven days at refrigeration storage. Commercialization of any technology depends on the ability to be preserved in its fresh form for longer time at retail outlets. With this objective attempts were made to extend the shelf life of sorghum based beverage. For this, preservatives namely Nisin, MicroGARD and Potassium sorbate were used before packaging of lassi. The product was packaged in LDPE pouches of 200 ml size. The product was stored at refrigeration temperature (6±1°C) and evaluated for sensory, physico-chemical and microbiological attributes at predetermined intervals. It was noticed that samples containing Potassium sorbate (P), Nisin (N) and MicroGARD (M) were found acceptable up to 35, 28 and 21 days respectively. For shelf life extension of pearl millet based lassi, MicroGARD and Nisin were used. The study revealed that product containing microGARD and Nisin were found acceptable up to 28 and 35 days, respectively (Hussain et al. 2012).

2.2 Tarhana

Tarhana is widely consumed traditional fermented food in Middle East countries and is of great importance in the diet of Turkish people. It is mainly used in the form of a thick and creamy soup. It is prepared by mixing wheat flour, yoghurt, yeast and a variety of cooked vegetables and spices such as tomatoes, onions, salt, mint and paprika). The mix is
fermented by yoghurt bacteria (Lactobacillus bulgaricus and Streptococcus thermophilus) and Baker’s yeast (Saccharomyces cerevisiae) for one to seven days resulting in acid production and leavening. The dough at fermentation is called as wet tarhana. Afterwards, the dough is dried to obtain dry tarhana (Figure 3). Tarhana has an acidic and sour taste with a strong yeasty flavor and is also a good source of proteins and vitamins (Gabrial et al 2010).

Production method and ingredients used in tarhana manufacturing may vary from region to region but cereals and yoghurt are always two of the major components. Tarhana can be divided into four categories based on its production method viz. “flour tarhana,” “goce tarhana,” “semolina tarhana,” and “mixed tarhana.” The difference lies with the use of wheat flour, chopped wheat, and semolina separately or as combinations while manufacturing. Apart from wheat flour, other cereal and legume flours such as rye, maize, barley, soybean, and chickpea can also be used in the production of tarhana (Kabak and Dobson, 2011).

![Flow diagram for manufacturing Ready to reconstitute bajra/sorghum lassi](image-url)
Onion, Tarhana herb, basil & dill, tomato paste and Paprika paste

Mixing

Pasteurization (65°C/ 30 min)

Wheat flour / yoghurt / salt

Cooling (25°C)

Baker’s yeast

Mixing and kneading to obtain Tarhana dough

Spreading dough on trays

1<sup>st</sup> incubation (30°C/24h)

2<sup>nd</sup> incubation (18-24°C/24h)

Dividing dough into big pieces

3<sup>rd</sup> incubation (18-24°C/24h)

Dividing dough into smaller pieces

Keeping at room temp. 24 h 4<sup>th</sup> day

Sieving

Conditioning at room temp. 24 h 5<sup>th</sup> day

Final drying process 35°C/48h

Grinding

Sieving (<1mm)

Packaging

Fig. 3 Flow Diagram for Manufacturing Tarhana

(Gabrial et al. 2010)
2.3 Kishk

Kishk is a traditional cereal based beverage popular in Middle East countries and it is prepared by fermentation of wheat-milk mixture. Wheat is added in the form of cracked and parboiled grains which is called as bulgur. Yoghurt is added to this bulgur and the mix is allowed to ferment at ambient temperature for different periods of time (Tamime and Connor, 1995). The wheat grains are boiled until soft, dried, milled and sieved in order to remove the bran. Milk is separately soured in a container, concentrated and mixed with the moistened wheat flour. The milk undergoes a lactic fermentation and the resulting paste is dried to a moisture content of 10–13% and then ground into a powder. The product is stored in the form of dried balls, brownish in colour with a rough surface and hard texture (Blandino et al, 2003).

3. Conclusion

Cereals are rich source of energy and nutrients but some coarse cereals like sorghum, pearl millet, barley, rye. Oat etc. lack some of the essential amino acids and at the same time, the products made thereof have poor sensory characteristics as compared to other cereals like wheat and rice and also to other foods of animal and plant origin. Numerous technological interventions have been attempted to increase the palatability and nutritional value of such coarse cereals. Fermentation is by far the most common and widely accepted technology for improving nutritional value and palatability. World over, a myriad of fermented cereal based beverages such as kishk, tarhana, raabdi, etc. are manufactured but their production is limited to household scale. Standardization of technology and manufacture of such products at industrial scale will not only provide consumers a healthy choice but also at affordable prices.

4. Suggested Reading


1. Introduction

Being as nature’s most complete food, milk is well known for its nutrient potent, physiological and biochemical functions played by its biologically active constitutes i.e. casein and whey protein that have crucial impacts on human metabolism and health. Recent research had revealed that milk is a source of several biologically active compounds which deliver safeguard to neonates and adults against both pathogens and disease. Milk proteins are usual vehicles, which developed to transport essential micronutrients and immune system components, from mother to the neonatal. Major milk proteins are shown in Table 1 (Linvey, 2010).

Table 1: Major Proteins in milk.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Content in milk (g/l)</th>
<th>Molecular weight (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caseins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>αs1 (αs1-CN)</td>
<td>12–15</td>
<td>22.1–23.7</td>
</tr>
<tr>
<td>αs2 (αs2-CN)</td>
<td>3–4</td>
<td>25.2–25.4</td>
</tr>
<tr>
<td>β (β-CN)</td>
<td>9–11</td>
<td>23.9–24.1</td>
</tr>
<tr>
<td>κ (κ-CN)</td>
<td>3–4</td>
<td>19.0</td>
</tr>
<tr>
<td>Whey proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-lactoglobulin (β-lg)</td>
<td>2–4</td>
<td>18.3</td>
</tr>
<tr>
<td>α-lactalbumin (α-la)</td>
<td>1–1.5</td>
<td>14.2</td>
</tr>
<tr>
<td>Bovine serum albumin (BSA)</td>
<td>0.1–0.4</td>
<td>66</td>
</tr>
<tr>
<td>Immunoglobulins (Ig)</td>
<td>0.6–1.0</td>
<td>146–1,030</td>
</tr>
<tr>
<td>Lactoferrin (Lf)</td>
<td>~0.1</td>
<td>80</td>
</tr>
<tr>
<td>Milk fat globule membrane (MFGM) proteins</td>
<td>~0.4</td>
<td>13–200</td>
</tr>
<tr>
<td>Total milk proteins</td>
<td>30–35</td>
<td></td>
</tr>
</tbody>
</table>

Source: Adapted from Linvey, 2010.

2. Hydrolysis and its importance

Protein being a high molecular weight compound made up from low molecular weight amino acids linked with peptide linkage plays a crucial role in our diet. Truly, proteins in its original intact form, holds some functional properties but its modified form being much better than its usual intact form in terms nutritional and functional properties. Protein hydrolysate/hydrolyzate is also one of the modified form of protein which is a rich mixture of amino-acids obtained via hydrolysis of protein using acid, alkali or by proteolytic enzymes as such (Kristinsson and Rasco, 2000) or secreted by certain groups of biological species. Proteolytic modification of food proteins for the betterment of the available protein resources is an ancient technology. In simple words, hydrolysates can be defined as “the proteins that had been chemically or enzymatically broken down into peptides of varying sizes” (Dave et al., 1991). Extent of hydrolysis or the amount of
protein breakdown into peptides and amino acids is expressed as % amino nitrogen/degree of hydrolysis (DH). Thus DH is the ratio of peptide bonds cleaved (total number) and the total number of peptide bonds present originally in the whole protein. In the manufacturing of protein hydrolysates, % DH is one of the essential controlling factors which reveal on the product superiority (Kanawjia et al., 2008). Protein hydrolysate, now a days being produced with wide variety of uses for different industries such as biotechnology, food & pharmaceutical industry (Kristinsson and Rasco, 2000). Currently, hydrolysis of milk proteins is the main pathway in the production of bioactive peptides and functional ingredients. It is also well known that patients with impaired luminal hydrolysis, weakened absorptive capacity and hepatic failure, unable to digest proteins in its native form. Similarly, allergenic patients, the administration of native or partially hydrolyzed protein can provoke immune intermediated hypertensive reaction. In such situations, either mixture of synthetic amino acids or protein hydrolysate can play a crucial role to supply tailored amount of amino acids (Clemente, 2000).

![Fig.1. Steps in acid hydrolysis of protein](adopted from Dave et al., 1991)

3. Methods and mechanism of hydrolysis

There three main methods for protein hydrolysis viz. (a) acid (b) alkali and (c) enzymatic hydrolysis.

3.1 Acid hydrolysis of proteins

The first reported acid hydrolysis goes back to 1820. Soy sauce was the first protein hydrolysate produced historically by cooking a blend of soybean and wheat together and allowing this mixture to become mouldy over a period of six months. Thus, hydrolysis was accomplished by the secretion of proteolytic enzymes from mold species. After several decades, acid hydrolysis was commercially used and still in practice as an older process. Although, hydrochloric and sulfuric acid are mainly used for the production of hydrolysates yet hydrochloric acid is preferred over sulfuric acid. During this process,
proteins break into individual amino acids and traces of smaller peptides. Process parameters which not only act as the governing factors for acid hydrolysis but also had significant impact on product quality are protein source and its purity, type and concentration of acid (HCl or H$_2$SO$_4$), temperature, pressure, duration and extent of hydrolysis. Outline of processes used in the production of acid protein hydrolysates are shown in Fig.1. Its main disadvantage includes destruction of essential amino acids such as tryptophan, methionine, cystine, cysteine and production of glutamic and aspartic acid from glutamine and asparagine.

3.2 Alkaline hydrolysis of proteins

It is a fairly straight forward and modest process. Alkaline hydrolysates of proteins are commercially used in food industry. During alkaline hydrolysis, tryptophan remains integrated but other amino acids like serine and threonine are destroyed. In this process, first proteins are solubilized by heat followed by addition of alkaline agents like calcium, sodium or potassium hydroxide and then temperature is kept constant to a desired set point. This process continued for several hours until it reaches the desired extent of hydrolysis.

3.3 Enzymatic hydrolysis of proteins

Most of the enzymes used to make protein hydrolysates are obtained from animal, plant and microbial sources. The main merit of enzymatic hydrolysis is offering mild hydrolysis conditions and strict control over the DH due to specificity of enzyme to get tailor make products. Different proteolytic enzymes are commercially available from animal (Pancreatin, Trypsin, Pepsin), plant (Papain, Ficin and Bromelain) and microbial (Neutrase, Alcalase, Esperase, Pronase, Naturage, etc.) sources. Desired extent/ degree of hydrolysis can be achieved using single enzyme in a single step or by sequential enzyme hydrolysis using multiple enzymes. Enzyme plays a crucial role in hydrolysis and selected on the basis of protein source and end user requirements (Pasupuleti and Braun, 2010). When compared with acid and alkaline hydrolysis of proteins, proteolytic enzymes offer following other advantages:

- Lesser amounts of enzymes are required that can be easily deactivated.
- It offers mild operating conditions like temperature and pH.
- Enzymatic hydrolysis results in higher retention of amino acids.
- A wide range of proteolytic enzymes are available i.e. manufacturers have choice to select the required enzyme that will best suits to their needs.

Exact mechanism involved in protein hydrolysis and precise control over process parameters remains unclear, their production is massive around the world. Hydrolysis of peptide bonds generally results in three major effects: (i) Increased hydrophilicity i.e. higher number of ionized groups ($\text{NH}_4$ and COO$^-$) higher is the hydrophilicity t; (ii) shorten size of polypeptide chains or reduction in molecular weight i.e. reduces protein’s antigenicity; and (iii) release of hydrophobic moiety hitherto concealed (Panyam and Kilara, 1996).

4. Generic flow for manufacturing protein hydrolysates

Type of product and scale of production determines the size production line. As shown in Fig. 2, its production steps starts with solubilization of proteins in water (8–20% solids, if required pretreated with heat/acid /alkali) followed by proper pH (3.5–9.0) and temperature adjustment. Suitable enzyme or multiple enzymes are than added to the reactor systems. The duration of hydrolysis may vary anywhere from 1 to > 100 h. In
order to check microbial contamination in reactors, chemical preservatives (bacteriostatic or bactericidal) are used which must be removed after hydrolysis. Currently, UV treatments, pulse electric field (PEF) treatments are being used for this purpose. When desired degree of hydrolysis (monitored by taking in-process samples) is reached, the hydrolysis process is terminated by deactivating enzyme through heating the solution. This digested solution is then subjected to a series of purifying steps depending on the end application of the product (i.e. Separation of insoluble from the digested solution by centrifuge / plate and frame filter press /MF system to get desirable color and clarity of the solution which is critical too). For the removal of coloring and haze-forming components, charcoal powder is effectively being used. The replacement of plate and frame filter press by UF systems (spiral wound ceramic or hollow fiber membranes with a MWCO of 10 K Da) which offers the removal of endotoxins is a newer addition to hydrolysates manufacturing. The solution is then pasteurized/heat treated to kill or reduce the MO’s and evaporated to increase its solids (30–50%). This concentrated solution is then dried in a spray drier and finally hydrolysate in its powder form, filled into boxes, bags or drums for packaging and stored (Pasupuleti and Braun, 2010).

5. Post-hydrolysis processes

Generally protein hydrolysis can’t offers fully desired product until their modifications after hydrolysis. Commonly hydrolysates are modified to control their molecular size and also to reduce bitterness. Their bitterness can be reduced or modified by several methods i.e. separation, extraction, precipitation and masking of bitter tasting compound, few such post hydrolysis methods used in their production are shown in Table 2.

6. Important points during production of protein hydrolysates

In order to produce high quality product following practices should be applied at production site: strict adaption of GMP, high degree of plant hygiene is essential, severe screening of raw materials to obtain consistent and high quality raw materials, uninterrupted monitoring in-process samples to continue uniformity of the lots, testing.
end product samples of every batch, constantly monitoring sources of protein, enzyme, water and the purifying techniques.

Table 2: Main post hydrolysis processes used in the development of protein hydrolysates.

<table>
<thead>
<tr>
<th>Post-hydrolysis Processes</th>
<th>Action</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrafiltration</td>
<td>Removal of high molecular weight peptides and proteins</td>
<td>Broad</td>
</tr>
<tr>
<td>Heat Treatment</td>
<td>Inactivation of proteolytic enzymes To improve the access of proteolytic enzyme</td>
<td>Broad Food allergy</td>
</tr>
<tr>
<td>Hydrolysis by exoproteases</td>
<td>Increase the extent of hydrolysis Decrease bitterness</td>
<td>Broad</td>
</tr>
<tr>
<td>Activated Carbon</td>
<td>Decrease bitterness</td>
<td>Broad</td>
</tr>
<tr>
<td>Ionic exchange resins</td>
<td>Selective loss of phenylalanine</td>
<td>Phenylketonuria</td>
</tr>
<tr>
<td>Use of specific enzymes</td>
<td>Decrease the content of specific amino acids Phenylyalanine Aromatic amino acids</td>
<td>Phenylketonuria Liver disease</td>
</tr>
<tr>
<td>Phenylalanine ammonia lyase, actinase, carboxypeptidase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absorption Chromatography</td>
<td>Decrease the content of aromatic amino acids</td>
<td>Liver disease</td>
</tr>
</tbody>
</table>


Table 3: Major biologically active milk components and their functions

<table>
<thead>
<tr>
<th>Milk Components</th>
<th>Bioactive compounds</th>
<th>Observed bioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-, β-caseins</td>
<td>Casomorphins, Casokinins, Phoshopeptides, Immunopeptides</td>
<td>Opioid agonist, ACE inhibitory, Mineral binding, Immunomodulatory</td>
</tr>
<tr>
<td>αs1-caseins</td>
<td>Isracidin</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>αs2-caseins</td>
<td>Casocidin</td>
<td></td>
</tr>
<tr>
<td>κ-casein</td>
<td>Casoxins, Casoplaterins, κ-casein glyco-macropeptide</td>
<td>Opioid antagonist, Antithrombotic</td>
</tr>
<tr>
<td>α-lactalbumin (α-La), β-lactoglobulin (β-La)</td>
<td>Lactorphins</td>
<td>Opioid agonist</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>Serorphin</td>
<td>Opioid agonist</td>
</tr>
<tr>
<td>α-La, β-La and Serum albumin</td>
<td>Lactokinins</td>
<td>ACE inhibitory</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>IgG, IgA</td>
<td>Immunomodulatory</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Lactoferrin, Lactoferroxins</td>
<td>Immunomodulatory, Opioid antagonist</td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>Oligosaccharides</td>
<td>Prebiotic (Increase growth of bifidobacteria in GI tract)</td>
</tr>
<tr>
<td>Glycolipids</td>
<td>Glycolipids, Oligosaccharides</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>Growth factors</td>
<td>IGF-1, TGF-α, EGF, TGF-β</td>
<td>Organ development and functions</td>
</tr>
</tbody>
</table>

Source: adopted from Young, 2010.
7. Applications of protein hydrolysates

Protein hydrolysates have a huge range of applications, e.g., they are an active ingredient in the formulation of medicine, specialist beverages with high nitrogen content, predigested nutrition for enteral/parenteral and general/specific population segments, energy drink, sport drink, diet of aged people and weight controlling diets, bacterial growth medium, ingredient of health care and cosmetic products and most important enriched/isolated peptide preparations for advantageous physiological function. The major biologically active milk components and functions in milk precursors and components are summarized in Table 3.

8. Selected Reading

1. Introduction

Since time immemorial the traditional Indian dairy products have been an integral part of the socio-cultural, nutritional and economical legacy of India. The mass appeal enjoyed by the indigenous sweets is underlined by the fact that about 50% of India’s milk production is utilized for making these products (Dutta and Bakshi, 2011). The total output of Indigenous milk products is estimated to be 12 billion USD. This is more than half of the total market (22 billion USD) of milk and milk products in India. Despite such a great role played by the traditional dairy products in national food economy, most of these products are still produced by small-scale traders or sweet meat makers, mostly employing age-old energy inefficient, labour intensive processes. The traditional dairy products present a great opportunity for the organized dairy sector in the country to modernize and scale up their production. Their production and marketing can bring about remarkable value addition to the extent of 200 per cent, as compared to only 50% obtained by Western products like butter, cheese and milk powders (Aneja et al., 2002). The consumption of traditional dairy products is likely to grow at an annual growth rate of more than 20%, but for Western dairy products the growth rates are relatively much lower, varying from 5-10%. Thus, the expanding business prospect provided by the traditional Indian dairy products to the organized dairy sector triggers a thorough face-lift of this sector.

2. Developments in production of traditional milk products

Attempts have been made by various Institutes of India to optimize production process, develop mechanized production lines and packaging systems to overcome the inherent disadvantages associated with the conventional methods of manufacturing of traditional dairy products. To impress upon the Indian Dairy industry, National Dairy Development Board set up an indigenous product plant in the late ‘70s known as “Sugam Dairy”. The plant started with mechanized gulabjamun and shrikand product lines with improved production protocol and packaging system. With considerable capital investment and packaging expenses, the dairy could compete with the local sweet shops in pricing. This experience encouraged a few organised dairies throughout the country to venture out in various traditional products and since then. The efforts that have been made mainly at NDRI, Karnal and NDDB, Anand have resulted in development of mechanized semi-continuous or continuous systems for mass production of some major indigenous milk products that could be adopted by the organized sector for mass production. Some of such systems have been described in the following section.

2.1 Khoa

Several attempts in past in development of khoa making process have been directed towards development of plant which would enable industrialization of khoa making process. The most successful innovation for continuous khoa making is the development of Inclined Scrapped Surface Heat Exchanger (ISSHE). In this machine khoa manufacturing involves concentrating milk through a milk-condensing unit, converting the condensed milk to khoa mass by the ISSHE, followed by cooling of khoa using a
rapid cooling unit. Milk concentrate used as feed is pumped into the ISSHE at the desired flow rate by adjusting the capacity of the feed pump. The inclination of the ISSHE, permits formation of a pool of boiling milk critical to formation of khoa. Subsequently, fresh concentrated feed enters the pool of boiling concentrated milk, while an equivalent mass continuously leaves the pool as semi solid mass. The scraper repeats the process of removing of coagulated particles from the heat transfer surface and mixing them back to the pool of heated milk. The coagulated particles absorb milk resulting in the agglomeration and formation of characteristic khoa texture. The inclination of the scraper provides interface between metal, milk and air, which enhances the heat coagulation of proteins. The system is shown in Figure 1.

Khoa mass comes out from the ISSHE at around 90°C. For cooling of khoa from 90°C to 25°C a Rapid Cooling System has been developed which operates on the principal of adiabatic flashing wherein khoa is exposed to high vacuum, resulting in evaporation of moisture of khoa which in turn facilitates reduction in temperature of the product. The ISSHE process is now being used on a regularly basis in several dairies in India and abroad.

A Three Stage Thin Film Scrapped Surface Heat Exchanger for continuous production of khoa has recently been developed at NDRI, Karnal. The system consist of three SSHEs connected in cascading mode, variable speed drives, balance tank, feed pump, sugar dosing device, steam supply valves and necessary controlling instrumentations. A schematic diagram of the system is presented in Figure 1A. All the three SSHEs are identical in length, diameter and effective heating length. The rotor assembly of first two heat exchangers is identical but different from the rotor assembly of third SSHE. The first and second stages SSHE had four variable clearance blades that are hinged between the cross supports in each scraper. The rotor of the third stage SSHE consists of two variable clearance blades and two skewed blades. The scraper assembly is mounted on a roller bearing housed in end cover of SSHE. The third stage SSHE is also provided with sugar dosing device. All SSHE’s jackets are provided with spring loaded safety valves and vent cock on different locations. The incorporation of the sugar dosing unit in the unit also enables the system to be utilized for the production of sugar containing concentrated or heat coagulated milk products like basundi, burfi and peda (Singh and Dodeja, 2012).
2.2 Gujabjamun

Gujabjamun is a khoa-based product, which is popular all over the country. Gujabjamun is shaped either round or cylindrical, has a golden brown colour and has a soft firm body and smooth texture. A semi-continuous process line has been developed for large-scale manufacture of Gujabjamun which in operation at Baroda District Cooperative Milk Producers’ Union Ltd., Vadodara, Gujarat. The process line in depicted in Figure 2. The raw materials; khoa, wheat flour and baking powder are mixed in a planetary mixer. The dough is then fed to a portioning machine, which is a modified meat ball manufacturing unit available in European countries. The portioned mass is shaped like cylinders in a rolling machine before going to a continuous ball frying machine containing oil at a

Figure 1A. Schematic diagram of Three Stage Thin Film Scrapped Heat Exchanger for Continuous Khoa Production (Singh and Dodeja, 2012)

Figure 2. Semi-continuous line for production of gujabjamun
temperature of 140°C. The balls after frying are fed to a sugar syrup soaking tank containing 62% sugar syrup wherein the fried balls are allowed to soak for about two hours. Ready gulabjamuns then go to canning and sealing machine. The gujabjamun line can produce up to 1800 kg of the product per day. Canned gulabjamuns are now being regularly exported to Gulf countries and the USA (Bandyopadhyay and Khamrui, 2007).

2.3 Peda

For peda preparation khoa is mixed with sugar approximately at 3:1 ratio and then heated in a shallow pan made of food grade metal. After heating, desired flavourings and nuts are added to the mass. The heating process removes some of the moisture of khoa. Sugar, along with providing sweetness, exerts preservative effect. According to industry estimates the peda production in India exceeds that of any other indigenous milk-based sweets. In order to undertake commercial production with extended shelf-life of peda a mixing, kneading cooking and peda forming unit has been developed.

2.4 Chhana

Chhana is a heat acid coagulated product, which forms the base of several popular Indian sweets such as rasogolla, sandesh, rasomalai, chhana podo, cham cham, etc. Prototype equipment for the manufacture of chhana with a capacity of 40 kg/hr has been recently developed. In the system, milk stored at refrigerated condition is heated to suitable temperature by a plate/helical coil heat exchanger, supplied to a column where it is coagulated by using citric acid solution. The process uses a duplex plunger pump for metering milk and acid solution to the unit. After mixing acid solution with the heated milk, milk-acid mixture is allowed to move through a vertical column. The coagulum from the discharge end of the column is held in a trapezoidal inclined chute before separating milk solids from the whey through a vacuum assisted inclined strainer. Vacuum is maintained under the strainer to remove whey from the coagulated mass. The system is illustrated in Figure 3. The unit facilitates continuous coagulation of milk. Steady and pulsating motion of milk-acid mixture ensures formation of lumps without breakage and recovery of more milk solids. The coagulated mass could be used as chhana for converting it to various sweets and also the same could be transferred to proper press for manufacturing paneer.
2.5 Rasogolla

Rasogolla is one of the most popular traditional milk delicacies and undoubtedly the king of Indian milk sweets. A mechanized system has been developed for continuous production of rasogolla ballas are now commercially available. The system comprise of a channa kneading cum proportioning, ball forming and cooking unit. The channa is put in to a hopper, the hopper has specially shaped vanes and cones, which thoroughly knead the channa into a homogeneous mass and push down with the help of screw. The portioned chhana falls into a conveyor belt. A combination of linear and rotating motions of the conveyor belt provides the ball forming action. The linear motion of the belt provides the forward motion of the balls, which falls into a cooking system, consists of a screw conveyor in a semi-cylindrical trough containing sugar syrup. A motor rotates the screw conveyor very slowly. This cause the balls gradually move forward while getting cooked in the syrup. The length and RPM of the screw conveyor are so adjusted that when the balls reach the exit side of the cooking unit cooking is completed.

2.6 Sandesh

Sandesh is one of the most popular chhana-based sweet of the eastern parts of India. Considering the huge domestic market of this product R & D studies have been undertaken for mechanization of sandesh production. A jacketed scrapped surface conical bottom vessel, which performs kneading, mixing, heating, and cooking operations involved in manufacture of sandesh has been developed. The unit consumes less time and offers high rate of heat transfer, proper utilization of energy and better control over process parameters in comparison to traditional method of preparation of sandesh.

2.7 Paneer

Paneer exalts a premier position amongst the Indigenous dairy products of India. The efforts of various Institutes has lead to the development of standardised manufacturing process for commercial production using semi continuous processing line. The process sequence is depicted in Figure 4. For paneer production buffalo milk is preferred to cow milk because cow milk produces soft, meaty texture of paneer, unsuitable for frying. High heat treatment (90°C/no hold) of milk improves the solids recovery, yield, flavour and body & texture characteristics of paneer. Coagulation at 76°C and pH 5.30-5.35 with one percent citric acid solution produces best body and texture characteristics. Within 10
minutes of coagulation, the curd chunks sink to the bottom of the cheese vat. After straining of the whey the curd is transferred to hoops, lined with cloths. Four to five paneer hoops, containing 10 kg of paneer, are placed one another and a pressure of 2-3 kg/cm² is exerted using hydraulic press. The pressing time is around 15-20 minutes. The paneer blocks are cut and cooled to about 4°C by immersing in chilled water.

3. Developments in shelf-life and packaging systems of indigenous dairy products

One of the main reasons that impede the Indian organized dairy sector to take up the commercial production of indigenous milk products has been the short shelf life of these products. Several attempts have been made to improve the shelf life of indigenous milk products by developing suitable packaging systems. Modified Atmosphere Packaging (MAP) and use of barrier film with oxygen scavengers have been introduced for shelf life extension of peda in a few dairies in India. Peda samples packed in barrier film with MAP comprising of 80% nitrogen and 20% carbon dioxide extended shelf life to 15 days at 37°C, and 30 days at 20°C of storage. Whereas, packaging peda in barrier film with oxygen scavengers extended the shelf life up to 2 months at 37°C, 5 months at ambient temperatures and 6 months at 20°C.

Products like rasogolla and gulabjamun are to be marketed dipped in sugar syrups as it is very important to retain their shape and body & texture. Tin can is the most suitable package for this product group. Packing Rasogolla in 4-mm thickness cans with inside tinning and lacquering with food grade epoxy phenolic compounds has shown shelf life of 60 and 140 days when stored at 30° and 7°C. To reduce the expense in tin cans rasogolla and gulabjamun are now being packed in metallised polyester or HDPE made “standi pouches”. However, as compared to tin cans the shelf life of the products are much shorter, with a maximum up to 28 days even at refrigerated condition of storage (Bandyopadhyay and Khamrui, 2003).

4. Conclusions

The traditional milk products of India have been plagued with various inherent problems largely affecting the organized sector to take up their production on industrial scale. Initiating commercial production of traditional dairy products by Indian dairy industry can do wonders for them in terms of financial stability and steady growth. However, in order to meet the quality standard of modern era, total mechanization of indigenous manufacturing process and development packaging system for enhancing the shelf life in the need of the hour.

5. Selected Reading


Preparation of Synbiotic Ice cream

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Dairy Technology Division

1. Introduction

With the start of this millennium, we witness a new era in the field of food science and nutrition with an increasing importance given to the interaction of food and medicine. The outcome of this area of study is known as “Functional Foods”, which involves food components as essential nutrients required for optimum health and as non nutritional component which contribute to the prevention or the delay of onset of chronic illnesses. The latest market surveys show that, in India, there is a great margin for such value-added health promoting food products.

Ice-cream is a food product highly acceptable by children, adolescents, and adults, as well as by the elderly public. Due to its refreshing traits, it is consumed more in the summer, but nowadays it is consumed throughout the year. Because of recent advances in production and rapidly developing technology, ice cream industry has become a profitable industry. Ice cream has become a high quality food due to the diverse ingredients and methods of freezing used in its manufacture and thus results in more than 200 different types of ice creams. Food Safety and Standards (Food Products Standards and Food Additives) Regulations (2011) defines Ice Cream as the product obtained by freezing a pasteurized mix prepared from milk and/or other products derived from milk, with or without the addition of nutritive sweetening agents, fruit and fruit products, eggs and egg products, coffee, cocoa, chocolate, condiments, spices, ginger and nuts and it may also contain bakery products such as cake or cookies as a separate layer and/or coating. The said product is frozen hard, has a pleasant taste and smells free from off flavor and rancidity, may contain food additives permitted in these regulations and must conform to the compositional and microbiological requirements specified.

2. Probiotic, prebiotic and synbiotics

2.1 Probiotics

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits on the host (FAO/WHO, 2001). Lactobacillus and Bifidobacterium are common species of bacteria used as probiotics for the production of dairy products (Fuller, 1989). Both viable and nonviable microorganisms could have probiotic properties, but viable cultures have better effects (Ouwehand and Salminen, 1998). The global market for probiotics is expected to record a CAGR (Compound Annual Growth Rate) of 17% during 2009 to 2014 (Kanade, 2010). Desired characteristics of a good probiotic are as follows (Dunne et al. 2001):

- Exerts a beneficial effect when consumed
- Non-pathogenic and non-toxic
- Contains a large number of viable cells
- Has the capacity to survive and metabolise in the gut
- Retains its viability during storage and use
- If incorporated into a food, it should have good sensory qualities
2.1.1 Probiotic organisms

Within the LAB group, the genus *Lactobacillus* is the most widely encountered probiotics (Chukeatirote, 2003); these include *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. fermentum*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, etc. Other probiotic organisms including *E. faecium*, *S. cerevisiae* and *Propionibacterium* have potential to be used in probiotic products (Shah, 2007). Probiotic products containing *L. acidophilus*, *Bifidobacterium* spp. and *L. casei* are becoming increasingly popular.

2.1.2 Probiotic foods

Probiotic food is a food product containing viable probiotic microorganisms in sufficient populations incorporated in a suitable carrier (Gibson and Roberfroid, 1995). The lactic acid bacteria (LAB) have long been used for the fermentation of milk (cheese, yogurt), meats (dry sausages), cereals (sourdough), vegetables (sauerkraut), and fruits (wine). The original aim of fermentation by LAB was to preserve foods by acidifying them, or to develop specific flavors or textures. The incorporation of bacteria of the intestinal origin into human diet corresponds to the emergence of a new generation of food products that use the beneficial effects of these bacteria. For humans to develop beneficial effects, ingestion of $10^6$ to $10^9$ viable cells per day is necessary (Lee and Salminen, 1995). In addition, to have better consumer acceptance, acceptable taste and attractive texture are essential for all dairy products, regardless of the “health message” associated with them (Saxelin *et al.*, 1999). The viability and metabolic activity must be maintained in all the steps of the food processing operation and they must be able to survive in the gastrointestinal tract to confer health benefits to the host (Sanz, 2007).

2.2 Prebiotics

A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host’s well-being and health (Roberfroid, 2007). As a functional food component, prebiotics, like probiotics, are conceptually intermediate between foods and drugs.

Generally, it is assumed that a prebiotic should increase the number and/or activity of bifidobacteria and lactic acid bacteria. The importance of the bifidobacteria and the lactic acid bacteria (LABs) is that these groups of bacteria may have several beneficial effects on the host, especially in terms of improving digestion (including enhancing mineral absorption) and the effectiveness and intrinsic strength of the immune system. To maximize effectiveness of bifidus products, prebiotics are used in probiotic foods. The three criteria required for a prebiotic effect are as follows (Gibson et al. 2004):

- Resistant to gastric acidity and hydrolysis by mammalian enzymes and GI absorption
- Can be fermented by intestinal microflora.
- Selectively stimulates the growth and/or activity of intestinal bacteria associated with health and wellbeing.

2.2.1 Sources of prebiotics

Traditional dietary sources of prebiotics include soybeans, inulin sources (such as Jerusalem artichoke, jicama, and chicory root), raw oats, unrefined wheat and unrefined barley. Some examples of prebiotics are oligosaccharides, hi-maize, fructooligosaccharides (FOS), inulin, etc. Several types of oligosaccharides have shown to be prebiotics using *in vitro* models and human trials. Oligosaccharides that have been studied include lactulose, fructo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, isomalto-oligosaccharides, gluco-oligosaccharides and xylo-
oligosaccharides (Olano-Martin et al., 2002). Hi-maize, derived from high amylase maize (corn) is also a prebiotic that improves the survival of some probiotic organisms through the digestive process. FOS also sometimes called oligofructose or oligofructan is a class of oligosaccharide used as an artificial or alternative sweetener and as prebiotic also. It exhibits sweetness levels between 30 to 50 percent of sugar in commercially prepared syrups and is produced commercially, based on inulin degradation or transfructosylation processes. Inulin is a polydisperse β (2→1) fructan (Coussement and Franck, 1993) and was identified from the root of *Inula helenum*. It is present in a wide range of plants, including common vegetables, fruits and cereals (Van Loo et al., 1999). Inulin has technological and nutritional benefits and also possesses prebiotic activity. It is regarded as an active food ingredient for functional foods and confirms to GRAS status (Kolbye et al., 1992). Prebiotic foods are food products that contain a prebiotic ingredient in an adequate matrix and in sufficient concentration, so that after their ingestion, the postulated effect beyond that of usual nutrient supplies is obtained (de Vrese and Schrezenmeir, 2001).

2.3 Synbiotics

Synbiotics are mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of selected live microbial strains in gastrointestinal tract (de Vrese and Schrezenmeir, 2001). A synbiotic product contains a beneficial agent in the small intestine (probiotic) and one for large intestine (prebiotic), the two thus act synergistically, hence synbiotics.

2.3.1 Synbiotic foods

The market for synbiotic food category continues to expand with growing consumer awareness about the role of diet in health maintenance (Stanton et al., 2001). Intake of synbiotics has been demonstrated to modify the composition of the gut microflora, restore the microbial balance and therefore have the potential to provide health benefits (Fuller, 1991).

3. Ice-cream as a carrier for probiotics and prebiotics

Ice cream can serve as a good carrier for the functional ingredients viz., probiotics and prebiotic resulting in the development of synbiotic ice creams.

3.1 Incorporation of probiotics into ice cream

Ice cream and frozen desserts are considered good carriers of probiotics but the effect of freeze stress on the viability of probiotic organisms during manufacture and extended storage must be considered. In addition to adding value to the product, probiotic culture in ice cream provides it with the advantage of being functional. Each process stage must be optimized during production of probiotic ice cream so as to guarantee functional properties in the product. There are two ways of adding cultures to ice cream: DVS (Direct Vat Set) type, for the direct addition to the product during its manufacture or using the milk as a substrate for fermentation. In the second case, to prevent any undesirable effect it is essential that the pH must be controlled during the fermentative process and also the temperature during storage. In addition to the increased sensitivity of probiotic strains to low pH values (4.0–4.5), negative effects on sensory acceptance of the product may be observed. Probiotic ice creams with fermentation as a step for their production are more acidic due to the production of lactic acid during the fermentation process. These changes might be undesirable, since ice-creams are not traditionally characterized as high acidic food products. This problem can be solved by stopping the fermentation at pH values ranging from 5.0 to 5.5 (Vardar and Oksuz, 2007).
3.1.1 *Challenges in addition of probiotics to food products*

To develop a functional food product with probiotics, there exist a number of technological challenges. Some of the important challenges that must be considered before addition of probiotics in food products (Champagne and Gardner, 2005) include:

1. The type or form of probiotic.
2. Amount to be added to have a beneficial effect.
3. Toxicity
4. Survivability of probiotic cultures during processing:
   - Type of substrate
   - Heating
   - Freezing
5. Determination of the concentration of the strains or cell populations
6. Stability of the probiotics during storage:
   - pH
   - Oxygen
7. Changes in sensory properties of the food product on supplementation with probiotic cultures

3.2 Incorporation of inulin into ice cream

Inulin and oligofructose are neither hydrolysed by the human digestive enzyme, nor absorbed in the upper part of the intestinal tract (Ellegard et al., 1997). Inulin works in synergy with most gelling agents e.g. gelatin, alginate and maltodextrins and also improves the stability of foams and emulsions (Franck and Coussement, 1997).

Addition of inulin or oligofructose to food increases the uptake of Ca present in the diet (Franck, 1997). Contrary to this, Yeung et al. (2005) observed prebiotics such as inulin and FOS as promising factors enhancing iron absorption. The addition of inulin stimulated the growth of L. acidophilus and B. lactis and resulted in improved viability of these organisms in probiotic ice cream (Akin et al., 2007). Therefore, ice cream can be used as a food carrier of probiotics without impairing its quality and it can also be successfully delivered as a symbiotic product. The general flow diagram for the preparation of Synbiotic ice cream is given in Fig. 1.

5. Suggested Reading


Way Forward with Intense Sweeteners for Diabetic Food Formulations

P. Narender Raju and Ashish Kumar Singh
Dairy Technology Division

1. Introduction
Worldwide non-communicable diseases such as obesity, diabetes, cardiovascular diseases and cancer have become major health problems due to changing lifestyle and dietary patterns among people. The World Health Organization indicated that worldwide approximately 1.6 billion adults (age 15+) and 20 million children under the age of 5 years were overweight and at least 400 million adults were obese in 2005 and projected that approximately 2.3 billion adults will be overweight and more than 700 million will be obese by the year 2015 (WHO, 2006). Further, recent estimations revealed that worldwide more than 220 million people have diabetes (WHO, 2009). In 2005, an estimated 1.1 million people died from diabetes, with the number likely to be doubled by the year 2030 (WHO, 2009). India has the largest diabetic population with one of the highest diabetes prevalence rates in the world (King et al., 1998; Bjroek et al., 2003). It is predicted that the Indian diabetic population would rise to more than 80.9 million by the year 2030 (King, et al., 1998). An Indian National Urban Diabetes Survey reported the average diabetes prevalence rate as 12.1% (Ramachandran, et al., 2001). However, there was a large regional variation and the prevalence rates varied from 9.3% in Mumbai to 16.6% in Hyderabad. Type-2 diabetes is a chronic progressive disease that requires lifestyle changes (Knowler et al., 2002), the key lifestyle interventions being physical activity and a nutritional plan with reduced caloric intake (Franz, 1997). The dietary factors such as high intake of fats, sugars, milk and its products and low intake of fruits and vegetables were ascribed for the role in the non-communicable diseases (Gupta et al., 2006). Being aware of the impact of high fat and high sugar on health, today’s health conscious consumer is looking for the low-fat, low-sugar or sugar-free dairy products. With the continuous invention of low-calorie and high-intensity sweeteners it has been possible to develop dietetic dairy products for the benefit of health conscious consumers in general and calorie conscious consumers in particular. In the present manuscript, technological developments in the manufacture of artificially sweetened dairy products for the management of diabetes and alike have been presented.

2. Intense sweeteners
Sweeteners elicit pleasurable sensations with or without energy and contribute to bulk and characteristic colour. These attributes are desired attributes and hence positively regarded qualities in food products. But, calorie conscious people in general and diabetics in particular need to control their diet by cutting down their sugar and calories intake. Sweeteners can be classified, based on their contribution towards energy, as nutritive and non-nutritive sweeteners. Nutritive sweeteners are those substances, which when consumed, not only provide sweet taste but also contribute 4 kcal per gram of substance. It includes sugar, honey, D-glucose, invert sugar, caramel, maltodextrin, high-fructose corn syrup and dextrose syrup. Low-calorie sweeteners are nutritive sweeteners that are relatively less sweet than sucrose and provide energy between 1 to 3 kcal per gram. Polyols are low-calorie sweeteners (about 2 kcal per gram) that occur naturally in a number of fruits, all vegetables, cereals, algae, mushrooms, seaweeds, etc. e.g. sorbitol, maltitol, lactitol and mannitol. Non-nutritive sweeteners are those sweeteners that offer
no energy such as aspartame, acesulfame-K, sucralose etc. The intensity of the sweetness of a given substance in relation to sucrose is made on a weight basis (Table-1).

### Table-1. Relative Sweetness of Sweeteners

<table>
<thead>
<tr>
<th>Sweetener</th>
<th>Approximate Sweetness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>1.0</td>
</tr>
<tr>
<td>Crystalline fructose</td>
<td>1.2 - 1.7</td>
</tr>
<tr>
<td>HFCS, 55%</td>
<td>1.0</td>
</tr>
<tr>
<td>HFCS, 90%</td>
<td>1.0</td>
</tr>
<tr>
<td>Hydrogenated starch hydrolysates</td>
<td>0.4-0.9</td>
</tr>
<tr>
<td>Lactitol</td>
<td>0.4</td>
</tr>
<tr>
<td>Trehalose</td>
<td>0.45</td>
</tr>
<tr>
<td>Isomalt</td>
<td>0.45-0.65</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>0.6</td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.7</td>
</tr>
<tr>
<td>Maltitol</td>
<td>0.9</td>
</tr>
<tr>
<td>Xylitol</td>
<td>1.0</td>
</tr>
<tr>
<td>Aspartame</td>
<td>180</td>
</tr>
<tr>
<td>Acesulfame potassium</td>
<td>200</td>
</tr>
<tr>
<td>Saccharin</td>
<td>300</td>
</tr>
<tr>
<td>Sucralose</td>
<td>600</td>
</tr>
<tr>
<td>Stevioside</td>
<td>300</td>
</tr>
<tr>
<td>Alitame</td>
<td>2000</td>
</tr>
<tr>
<td>Neotame</td>
<td>8000</td>
</tr>
</tbody>
</table>

3. Alternatively sweetened dairy products

The dairy industry has responded to the growing needs of health conscious consumers for low-calorie foods. Consequently, a large number of dairy products made with low-calorie and/or non-nutritive sweeteners have been developed and some of them can be witnessed in the supermarket shelves. Some of the R&D efforts in this area are discussed here.

3.1 Ice-cream and frozen desserts

Olsen (1989) suggested an ice cream formulation with low fat and low sugar content having 3% fat, 0% sugar, 4% glucose syrup, 3% bulking agent, 0.05% aspartame and 0.7% stabilizer/emulsifier. Palumbo, et al. (1995) developed aspartame sweetened ice cream and ice milk bulked with lactitol and/or polydextrose. Olinger and Pepper (1996) described a process for frozen dessert sweetened with acesulfame-K in combination with lactitol and hydrogenated starch hydrolysate was used as the bulk sweeteners. Taste, texture, hardness, melting and overrun properties of the frozen dessert were reported to be comparable to those in conventional products sweetened with sucrose and corn syrup. Verma (2002) had developed frozen dessert using artificial sweeteners and reported that amongst the various sweeteners attempted, aspartame produced the most acceptable product. Further, it was reported that such frozen dessert contained 5.5% fat, 12.5% MSNF, 9.9% maltodextrin, 9.3% sorbitol, 1.5% WPC, 0.38% stabilizer and emulsifier and 400 ppm aspartame. Basyigit, et al. (2006) developed a human-derived probiotic ice cream using sucrose and aspartame and reported that the probiotic cultures remained unchanged in ice cream stored for 6 months regardless of the sweeteners used.
3.2 Fermented dairy products

Pinheiro, et al. (2005) reviewed the effect of different sweeteners in low-calorie yogurts. Keller, et al. (1991) had formulated an aspartame-sweetened frozen dairy dessert with increased MSNF but without bulking agents by treating it with lactase. It was reported that there were no significant differences in the scores of lactase-treated and artificially sweetened frozen desserts. Malone and Miles (1984) was granted a patent by the US patents organization for the development of a gelled, artificially sweetened yogurt prepared by mixing a stabilizer solution containing high methoxyl pectin (2-7%), low methoxyl pectin (3-8%) and an aspartic acid-based sweetener (0.1-0.75%). Farooq and Haque (1992) developed a non-fat low-calorie yogurt using aspartame and sugar esters and reported that sugar esters had improved the overall quality of non-fat low calorie yoghurt. It was reported that yoghurt with sugar esters, mainly stearate-type yoghurt with an HLB range of 5 to 9, had firmer body, texture, and mouth feel than yoghurts without sugar esters. Further it was reported that skim milk yoghurts sweetened with aspartame had 50% fewer calories per serving than regular yoghurt containing 3.25% fat and 4% sucrose. Keating and White (1990) had developed plain and fruit-flavoured yogurts using 9 different alternative sweeteners including aspartame, sodium and calcium saccharins, and acesulfame-K. It was reported that among all the plain and fruit flavoured yoghurts, yoghurts sweetened with sorbitol and aspartame received highest sensory flavour scores. Fellows, et al. (1991) developed a sundae-style yogurt using aspartame and reported that during the manufacture, aspartame has excellent stability in fruit preparation.

3.3 Traditional Indian dairy products

Burfi, the most popular khoa based confection contains high amounts of fat (20%) and sugar (30%). Prabha and Pal (2006) developed a technology for the production of dietetic burfi for a target group of obese and diabetics and reported that aspartame and neotame showed poor stability in dietetic burfi while sucralose provided the most desirable sweetness profile and excellent stability to the product. Recently, Arora et al. (2010) reported that aspartame sweetened (0.065%) burfi resembled control burfi in sweetness with 94% recovery of aspartame when stored at 6-8°C for 7 days. Chetna et al (2004) optimized conditions for making alternatively sweetened gulabjamun, another khoa based sweet, and reported that soaking of fried gulabjamun balls in sorbitol syrup of 54°B strength added with aspartame @ 0.25% maintained at 65°C for 3 h yielded a good quality product. Technology has been developed for the manufacture of sugar free rasogolla using artificial sweeteners for such a large group of people. The use of 40% sorbitol and 0.08% aspartame was found to be optimum for cooking of rasogolla balls. The higher sorbitol level resulted in hard body and unacceptable flavour where as lower level caused flattening of rasogolla balls with surface cracks.

Misti dahi contains high fat (1-12%) and cane sugar (6-25%) contents. High fat and sugar contents in misti dahi may pose a hurdle for its successful marketing in other parts of the country in the present health foods regime. With an aim to completely replace cane sugar in misti dahi, Raju and Pal (2011) attempted a blend of artificial sweeteners viz. aspartame and acesulfame-K along with different bulking agents and reported that maltodextrin was found to be the most suitable bulking agent. Further attempts to characterize the artificially sweetened misti dahi revealed that hardness and lightness were the most affected properties by the different binary blends of artificial sweeteners and bulking agents (Raju and Pal, 2012). Shrikhand has very high content of sugar (≥40). The effect of sugar replacers on sensory attributes and storage stability of shrikhand was studied by Singh and Jha (2005). Among various combinations of sugar and raftilose
tired, shrikhand prepared with raftilose (4%) and sugar (12.5%) was rated as most acceptable by the sensory panelists. Sugar and raftilose exhibited significant effect (p<0.01) on flavour, body and texture and overall acceptability no significant effect was observed on color and appearance.

Kumar (2000) developed a low calorie lassi, a traditional fermented refreshing beverage, by using aspartame and reported that aspartame at a level of 0.08% was required to replace 15% of cane sugar in lassi. Recently, George et al. (2010) studied the stability of multiple sweeteners in lassi and reported that binary blend of aspartame and acesulfame-K was found to be the best as it resembled control sample in all the sensory attributes up to 5 days of storage. Beukema and Jelen (1990) studied the suitability of developing whey-based drinks using high potency sweeteners and reported that both aspartame and acesulfame-K may be suitable sweetening agents in cottage cheese whey based fruit drinks. It was further reported that, in such drinks, the total calories were reduced to almost 50%.

4. Conclusion
With growing evidence of the role of diet and dietary components especially sugar in non-communicable diseases such as obesity and diabetes, worldwide people are cautious of what they eat. With the continuous invention of food additives such as low-calorie and high-intensity sweeteners it has been possible to develop dietetic dairy products that suit the palate of local consumers. R&D efforts in India contributed for the development of low-calorie dairy products such as dietetic rasogolla, burfi, misti dahi, kulfi, etc. for the benefit of health conscious consumers in general and diabetics in particular.

5. Suggested Reading


Research and Developments in Space Foods

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1. Introduction

From the beginning of human space travel, food has been an important feature that has involved astronauts, technicians, and engineers. The supply of food must be nourishing and tasty so astronauts maintain their health during their important stays in space. John Glenn, America's first man to eat anything in the near-weightless environment of Earth orbit, found the task of eating fairly easy, but found the menu to be limited. Failure to provide the adequate, nutrient dense food will hamper mission success and/or threaten crew performance. NASA needs research advances to develop food that is nutrient-dense and long-lasting at ambient conditions, partial gravity cooking processes, methods to deliver prescribed nutrients over time, and food packaging that meets the mass, barrier, and processing requirements of NASA.

2. Developments in space food

NASA food system literature and numerous publications have documented the evolution of the space food system (Huber and others 1972; Bourland 1993; Perchonok and Bourland 2002; Perchonok 2007). Even though significant development has transformed the space food system over the last 5 decades to attain more appealing dietary fare for low-orbit space crews, the advances do not meet the need for crews that might travel to Mars and beyond. It is estimated that a food system for a long-duration mission must maintain organoleptic acceptability, nutritional efficacy, and safety for a 3 to 5 years period to be viable.


In the early days of the space program, known as Project Mercury, space flights lasted from a few minutes to a full day. Because of the short duration, complete meals were not needed. The major meal was consumed prior to the flight. However, the Mercury astronauts did contribute to the development of space food. They tested the physiology of chewing, drinking, and swallowing solid and liquid foods in a microgravity environment. These first astronauts found themselves eating bite-sized cubes, freeze-dried foods, and semi-liquids in aluminum toothpaste-type tubes. The food was unappetizing, and there were problems when they tried to rehydrate the freeze-dried foods.

During the later Mercury test flights, bite-sized foods were developed and tested. These were solid foods processed in the form of compressed, dehydrated bite-sized cubes. The cubes could be rehydrated by saliva secreted in the mouth as food was chewed. Foods floating about in a microgravity environment could damage equipment or be inhaled; therefore, the cubes were coated with an edible gelatin to reduce crumbling. These foods were vacuum-packed into individual serving-sized containers of clear, four-ply, laminated plastic film for storage. This packaging also provided protection against moisture, loss of flavor, and spoilage.

The major advancements in food items during the Gemini period were more variety and improved packaging. The advances in dehydration process (Freeze drying) provided foods that were similar in appearance including color, taste, shape, and texture to freshly prepared food products. Gemini astronauts had such food choices as shrimp cocktail, chicken and vegetables, butterscotch pudding, and apple sauce, and were able to select meal combinations themselves.

5. Project Apollo (1968 – 1972)

The objective of this project was to land Americans on the Moon and return them safely to earth. By the time of the Apollo program, the quality and variety of food increased even further. Apollo astronauts were first to have hot water, which made rehydrating foods easier and improved the food's taste. These astronauts were also the first to use the "spoon bowl," a plastic container that could be opened and its contents eaten with a spoon. The moisture content allowed the food to cling to the spoon, making eating more like that on Earth. Another new package, the wet pack or thermostabilized flexible pouch, required no water for rehydration because water content was retained in the food. With these new pack-ages, Apollo astronauts could see and smell what they were eating as well as eat with a spoon for the first time in space. Some of the foods consumed on Apollo were coffee, bacon squares, cornflakes, scrambled eggs, cheese crackers, beef sandwiches, chocolate pudding, tuna salad, peanut butter, beef pot roast, spaghetti, and frankfurters.


The dining experience on Skylab was unique, unlike any other space flight. The Skylab laboratory had a freezer, refrigerator, warming trays, and a table. Eating a meal on Skylab was more like eating a meal at home. The major difference was the microgravity environment. Skylab foods were packaged in specialized containers. The rehydratable beverages were packaged in a collapsible accordion-like beverage dispenser. All other foods were packaged in aluminum cans of various sizes or rehydratable packages. To prepare meals, the Skylab crew placed desired food packages into the food warmer tray. This was the first device capable of heating foods (by means of conduction) during space flight. Because Skylab was relatively large and had ample storage area, it could feature an extensive menu: 72 different food items. Foods consisted of products such as ham, chili, mashed potatoes, ice cream, steak, and asparagus.

7. Shuttle/Mir and International Space Station (1995- till date)

For the Space Shuttle program, a more Earth-like feeding approach was designed by updating previous food package designs and hardware items. Rigid square rehydratable packages were being used but proved cumbersome and problematic on longer missions. Packages made of a lighter flexible material were developed and first tested on STS-44(1991). These Extended Duration Orbiter (EDO) packages are made of flexible plastic and have a valve for inserting water. These eventually replaced the rigid square rehydratable packages on a permanent basis. In addition, a trash compactor was developed to reduce the volume of the trash, and the new packages were designed to be compatible with the compactor. Diets are designed to supply each Shuttle crew member with all the Recommended Dietary Allowances (RDA) of vitamins and minerals necessary to perform in the environment of space. Caloric
requirements are determined by the National Research Council formula for basal energy expenditure (BEE).

For women, BEE = 655 + (9.6 x W) + (1.7 x H) - (4.7 x A), and
For men, BEE = 66 + (13.7 x W) + (5 x H) - (6.8 x A),
Where W = weight in kilograms, H = height in centimeters, and A = age in years.

8. Types of space food

There are eight categories of space food.

8.1 Rehydratable Food

The water is removed from rehydratable foods to make them easier to store. Water is replaced in the foods before they are eaten. Shuttle orbiter fuel cells, which produce electricity by combining hydrogen and oxygen, provide ample water for rehydrating foods as well as drinking and a host of other uses. Foods packaged in rehydratable containers include soups like cream of mushroom, casseroles like macaroni and cheese, chicken and rice, appetizers like shrimp cocktail, and breakfast foods like scrambled eggs and cereals.

8.2 Thermostabilized Food

Thermostabilized foods are heat processed to destroy deleterious microorganisms and enzymes so they can be stored at room temperature. Most of the fruits and fish (tuna fish) are thermostabilized in cans. The cans open with easy-open pull tabs similar to fruit cups that can be purchased in the local grocery store. Puddings are packaged in plastic cups.

8.3 Intermediate Moisture Food

Intermediate moisture foods are preserved by taking some water out of the product while leaving enough in to maintain the soft texture. Water is removed or its activity restricted with a water-binding substance such as sugar or salt. Intermediate moisture foods usually range from 15 to 30 percent moisture, but the water present is chemically bound with the sugar or salt and is not available to support microbial growth. Dried peaches, pears, and apricots, and dried beef are examples of this type of Shuttle food.

8.4 Natural Form Food

These foods are ready to eat and are packaged in flexible pouches. Examples include nuts, granola bars, and cookies.

8.5 Irradiated Food

Beef steak is the only irradiated product currently used on Shuttle. These products are cooked and packaged in flexible foil pouches and sterilized by ionizing radiation so they can be kept at room temperature. Other irradiated products are being developed for the ISS.

8.6 Frozen Food

These foods are quick frozen to prevent a buildup of large ice crystals. This maintains the original texture of the food and helps it taste fresh. Examples include quiches, casseroles, and chicken pot pie.

8.7 Fresh Food
These foods are neither processed nor artificially preserved. Examples include apples and bananas.

8.8 Refrigerated Food

These foods require cold or cool temperatures to prevent spoilage. Examples include cream cheese and sour cream.

9. Space food research and development

Foods flown on space missions are researched and developed at the Food Systems Engineering Facility (FSEF) at the NASA Johnson Space Center. The FSEF is staffed by Food Scientists, Dietitians, and Engineers who support both the Shuttle and Space Station food systems. Foods are analyzed for use on the Shuttle through nutritional analysis, sensory evaluation, freeze drying, rehydration, storage studies, packaging evaluations, and many other methods. Before any food takes flight though, it must be tested by the FSEF personnel on the NASA Zero-gravity KC-135 airplane, affectionately known as the "Vomit Comet" to see how the food item will react in micro-gravity. A food item is added to the menu only after it has undergone all the necessary research and development, and is approved for flight.

10. Challenges and current research in developing space food system

The following are the major technological and development needs for the space food system to successfully supply long exploration missions:

- Nutrient-dense, shelf stable foods that meet overall sensory acceptability metrics;
- Shelf stable menu items with at least a 5 years shelf life;
- Sustained vitamin delivery in shelf stable foods;
- Packaging material that meets high-barrier, low-mass, and process-compatibility constraints.

11. Nutrient dense food

In “Packaged Food Mass Reduction Trade Study,” Stoklosa found that significant reductions in the space food system mass are possible with further menu development (2009). The aim of the 1st part of the study was to maintain the overall number of calories provided to the crew but to increase the caloric density of menu items by maximizing the percentage of energy from fat (35% of total energy intake per NASA dietary guidelines). By optimizing the food system to have a 10% decrease in moisture and an increase in energy sourced from fat to 35%, a mass savings of 321 g per crew member per day, or 22%, is possible. The 2nd part of the study, which examined the substitution of standard menu items with one meal replacement bar per crewmember per day, resulted in a mass reduction of 240 g, or 17%.

12. Extended shelf life products

Food quality is predicted to have a pronounced role in crew psychological well-being due to the isolation and confined space associated with extended missions (Evert and others 1992). Shelf life study conducted at Johnson Space Center (JSC) from 2003 to 2008 highlighted the quality changes of the thermostabilized space foods over a 3 year period (Perchonok and others 2003; Perchonok 2005a; Perchonok and Antonini 2008). The shelf life study began with 13 thermostabilized items stored at 4.4°C (control), 22°C (storage temperature of actual flight food), and 35°C (accelerated temperature) and 50% relative humidity. Meat products were projected to maintain product quality the longest, over 3 years, without refrigeration.
Fruit products and dessert products followed, as they were projected to maintain their quality from 1.5 to 5 years without refrigeration. Starches and vegetable side dishes should maintain their quality from 1 to 4 years without refrigeration. Egg products did not respond adequately to the thermostabilization process and were found unsuitable immediately after production.

13. Vitamin delivery

Without adequate nutrition, human performance and sustainment are endangered. Zwart and others (2009) studied vitamin degradation during storage, noting significant decreases in folic acid, thiamin, vitamin A, vitamin C, and vitamin K in various space food products. The multivitamin tablet also underwent chemical degradation evidenced by decreases of 10% to 35% in riboflavin, vitamin A, and vitamin C by the end of the study. Encapsulated vitamin fortification or new methods of vitamin stabilization may be required to achieve nutrient-rich foods with limited degradation potential.

14. Optimized packaging for space food

During the development of an extraterrestrial food system, mission resources, including mass, volume, power, crew time, and waste disposal capacity, must be considered. Misuse of these resources could limit mission success. The major drawback of the current packaging system with regard to mass and volume as two different packages are actually being used jointly to pack a number of the products. The packaging materials used for the thermostabilized, irradiated, and beverage items contain a foil layer to maintain product quality beyond the required 18-m shelf life. Foil layer is not compatible with some emerging technologies foil packaging complicates plans to incinerate trash in the future and also metalized films generally do not provide the transparency necessary for the human inspection of products after packaging. As a part of the Advanced Food Technology study Total Systems Approach, a gusseted pouch design was compared to the current thermoformed rehydratable package (Oziomek 2010). The gusseted pouch design yielded very good results by making the rehydratable packages easier to produce, while minimizing mass, volume, and waste. It reduced the equipment required in the production process from 3 pieces of packaging equipment to one. It reduced the packaging from 2 pouches to one and decreased the total amount of packaging mass by about 66%.

15. Conclusion

Without an adequate food system, it is possible that space crewmembers health and performance would be compromised. It is clear that in developing adequate NASA food systems for future missions, a balance must be maintained between use of resources (such as power, mass, and crew time), and the safety, nutrition, and acceptability of the food system. In short, the food must provide the nutrients to sustain crew health and performance, must be acceptable throughout the course of the mission, must be safe even after cooking and processing, and must be formulated and packaged in such a way that the mass and volume are not restrictive to mission viability.

16. Selected Reading

Advances in Membrane Processing for Production of Novel Dairy Ingredients

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1. Introduction

Dairy ingredients are preferred ingredients for their functional supremacy and good flavour, colour and nutritional profile. Production of novel dairy ingredients through membrane processes that can be used in different foods based on their functional properties is now largely spread out, particularly after the commercialization of high mechanical and physico-chemical resistant mineral membranes. Membrane processes are increasingly being used because of their several inherent advantages like energy efficient process; easy, simple and economical operation; improved recovery of constituents and better yield of products and minimum thermal degradation problems, resulting in better nutritional and functional properties of milk constituents.

The main membrane systems in ascending order of pore size are: reverse osmosis (RO), nonofiltration (NF), ultrafiltration (UF) and microfiltration (MF). The distinction between the various membrane processes is somewhat arbitrary and has evolved with usage and time. In a broader sense RO is essentially a dewatering technique, NF a demineralization process, UF a method for fractionation and MF a clarification process.

Ideally, RO membranes should retain all components other than the water itself. RO has widely been used to concentrate dairy fluids and also make further products like khoa (Kumar and Pal, 1994) and condensed, concentrated and dried products. RO processed milk has also been successfully used in the manufacture of yoghurt, dahi, ice cream and several other dairy products. The principal application of NF is for separation of mineral ions in the $10^{-9}$ m size. The main emerging application of NF in the dairy industry is in partial demineralization of whey. Electrodialysis is a demineralization process based on the removal of charged mineral ions from the non-charged material. This process has wide application in demineralization of whey for use in many special dietetic foods including infant formulae.

UF membranes retain only macromolecules or particles larger than 1-20 nanometers. During UF process, a portion of lactose and minerals is removed. One of the major benefits of UF technology is its ability to retain whey proteins that are normally lost in whey in traditional manufacturing processes of cheese, chhana, paneer. UF membranes reject fat and all true proteins in milk. The proteins can be further concentrated by progressive removal of lactose and ash by a subsystem called diafiltration. During the last over four decades, UF technology has increasingly being used in the dairy industry because of many inherent advantages viz. saving on energy, improved yield of protein, enhanced nutritive value of the product and availability of a lactose stream in the form of permeate.

MF processes are designed to separate particles in the micrometer range (0.1-10 micrometers). This process retains fat globules, microorganisms and somatic cells, but allows passage of proteins in addition to lactose and minerals. The application of MF to skim milk in combination with other membrane processes and/or chromatography processes opens
up possibilities in isolating and purifying caseins and the peptides derived from them that can find application in the pharmaceutical industries.

Recent advances in membrane composition, configuration and porosity now allow microfiltration (MF) and ultrafiltration (UF) membrane systems to be used directly for individual milk protein fractionation having unique functional and nutritional characteristics. Fractionated milk protein isolates are finding increasing applications in infant foods formulations and dietetic products. The health and medical benefits of individual whey proteins are gaining acceptance at an increasing rate. Isolation of whey protein fractions can help in preparation of infant formulae with protein compositions more reflective of human milk. Dietary whey protein significantly retards the development of colon cancer and thus has potential as a functional food ingredient. The new generation infant formulas will require increase in the $\alpha$-lactalbumin and lactoferrin contents to get protein compositions similar to that of human milk.

Enzymatic modification of milk proteins permits development of peptides having unique physico-functional properties of pharmacological significance. These bio-peptides have been implicated in physiological roles such as biotransfer of trace elements, immunomodulation, antihypertension, antithrombosis, regulation of the gastrointestinal tract and the general behaviour (Morphine like activity). UF technology is being used as the most appropriate tool for separating low molecular weight peptides and free amino-acids from protein substrates utilizing enzymes.

2. Insoluble and soluble components of milk

Milk can be separated into its insoluble (caseins and fat) and soluble (whey proteins, lactose, peptides and NPN) components. This separation is advantageous in the sense that the casein and fat fraction can be processed separately, avoiding the inevitable interaction between the casein and whey protein fractions under the influence of heat. This approach allows reconstitution of milk with “bio-protective factors” intact. Alternatively, casein and fat rich retentate can be used for cheese production and the whey protein permeate can be ultrafiltered to obtainundenatured whey protein isolates of extremely high purity (pharmacological grade) displaying prophylactic quality.

3. UF milk retentate

UF milk retentate has widely been used for the manufacture of cheese and other fermented short shelf-life products where protein increase is desirable but lactose and ash increase is not desirable (Darghn and Savello, 1990; Green, 1990, Singh et al., 1994). In the Indian context UF retentate seems to be a highly promising base for long-life paneer (Rao, 1991; Singh et al., 1994). UF milk retentate has also been used to produce low lactose powder, non-dairy whitener, rasogolla mix powder, cheese base and milk protein concentrates.

Cheese base is a paste of the same composition and pH as Cheddar cheese but without the Cheddar flavour and structure. It is used to replace the young cheese component for the manufacture of processed cheese. For the production of cheese base, Milk is pasteurized, standardized to 3.8% fat, Cooled to 50°C, ultrafiltered to 30% TS, diafiltered to reduce lactose to desired level, further ultrafiltered to 40% TS, re-pasteurized, Cooled and 1% Cheddar starter culture added and evaporated to 60% TS. Processed cheese is made with blending cheese base (30%) with 70% normal aged Cheddar cheese.
Milk protein concentrates (MPC) is a relatively new dairy ingredient based on ultrafiltration and drying of skimmed milk. Typically with a protein content from 50-85% of total solids, MPC can be considered as a functional ingredient to be used in the manufacture of other foodstuffs. The main application of MPC today is in spreads and dressings. It is also used as a protein base in processed or even recombined cheeses. This high protein and high calcium ingredient can be used for the preparation of many dietetic foods including foods for elderly people and sport persons.

4. Condensed and dried whey products

By far the single largest use of whey solids on global basis is in the form of whole dry whey and it continues to grow. This is whole whey that has been condensed and spray dried as such or after blending with certain other liquid ingredients. These powdered whey products are marketed as commodity ingredients to be used in the manufacture of confectionery, chocolates, biscuits, breads and in the manufacture of animal feed.

A major problem with many whey-based products is their salty flavour owing to their high mineral content. Demineralized sweet whey (25-65% demineralization) can be used in foods such as coffee whitener, soft serve ice cream, milk shakes, whey drinks and caramel, citrus drinks, salad dressing, animal feeds, bakery goods, confectionery coatings and dry mixes. A range of demineralized spray-dried, whey-based products for use in infant feeds and dietetic applications have been developed based on products manufactured by demineralization and ultrafiltration of whey.

5. Native casein

A promising application of MF (0.2 µm pore size) of skim milk has been the selective separation of native casein micelles from the whey proteins (Sachdeva and Buchheim, 1997). If diafiltration with water is used during MF concentration, purified phospho-caseinate is obtained (Up to 90% protein in dry matter). After spray drying, such a product will easily compete with traditionally made calcium caseinates for many applications. Native casein has excellent rennet coagulating abilities and forms stronger gels at acidic pH (Famelart et al., 1996).

6. Casein fractions

Membrane MF (0.2 µm pore size) allows separation of β-casein by solubilization from calcium caseinate at 5°C and from skim milk at 4°C and pH 4.2-4.6. Products obtained on both sides of the MF membrane are suitable for modifying the β-casein/αs casein ratio of cheese milks and consequently, texture and flavour of resulting cheeses. New cheese varieties could be created in this way. The main interest in β-casein is related to the presence of peptides with biological activities in its sequence. Indeed, β-casein peptides are thought to be involved in bio-availability of trace elements, in morphinomimetic, cardiovascular and immunostimulating activities, not only for the young but also for adults and pregnant women.

7. Whey protein concentrates

UF process is now a major means of WPC production throughout most of the dairy countries of the world. WPC with 35% protein is perceived to be a universal substitute for NFDM, because of the similarity in gross composition and its dairy character. WPC can
also be seen competing with casein, egg albumin and soya proteins within the existing markets. Dietary whey proteins significantly retard the development of colon cancer and thus have potential as a functional food ingredient. Microfiltration of whey to obtain delipidised whey protein concentrates with improved properties has recently been tried out using different approaches (Gesan et al., 1995; Karleskind et al., 1995).

Due to various reasons, buffalo and cow milks are being humanised and used partly or exclusively for feeding human infants throughout the world. For humanisation, apart from making other modifications, whey proteins proportion needs to be increased in these milks. For this, a great potential lies in the application of WPC.

Whey protein based fat mimetics have recently been used in low fat variants of frozen desserts, yoghurt, fat spreads and cheese. These fat mimetics are made from concentrated cheese whey by special thermal and mechanical treatments which result in a controlled globular aggregation of denatured whey proteins termed as microparticulation (Buchheim and Hoffmann, 1994). Suspensions of such microparticles, with diameters in the range of about 0.1 to 3 μm can produce a creamy texture similar to that of globular fat particles, like the milk fat globules.

A special groups of ingredient called whey protein texturizer has been developed (Thompsen, 1994), that have unique properties of improved emulsifying property than whey protein concentrates, form gel without heat giving highly viscous solutions and give a firm gel upon heating. These can find application in comminuted meat products, mayonnaise, salad dressing and spreads, bakery products, French fries and potato products. These can also be used for preparations most suitable for heat induced structuring processes - thermoplastic expansion, microwave expansion and thermogelation. Such textured products act as extenders in various meat products.

8. Whey protein fractions

The health and medical benefits of individual whey proteins are gaining acceptance at an increasing rate. Isolation of whey protein fractions can help in preparation of infant formulae with protein compositions more reflective of human milk. From the defatted WPC, it is possible to prepare purified β-lactoglobulin and α-lactalbumin. β-Lactoglobulin is the protein which is essentially responsible for the gelling property of whey protein concentrates. β-Lactoglobulin could also have essential physiological activity as in the transport of vitamin A. This activity exists in most milk-producing animals but not in humans.

The α-lactalbumin has a great potential market. This protein is remarkably rich in tryptophan (4 residues per mole, about 6%); such a protein could allow preparation of tryptophan containing peptides which could have physiological properties. α-Lactalbumin is one of the main protein of human milk, so production of a formula suitable for infants could be based on native phosphocaseinate, purified α-lactalbumin and milk ultrafiltrate. Such an infant formula will have very low or may be no allergenicity. Although it has not been clearly explained, the high α-lactalbumin content of human milk might be indicative of the physiological role of this protein.

Defatted WPC is a very good raw material for the extraction of lactoferrin (Lf) and lactoperoxidase (Lp) through ion-exchange chromatography. Both Lf and Lp are already industrially produced, and are used in veterinary medicine to prevent diarrhea in young calves, in human eye drops, in mouthwashes, in chewing gum, and in infant foods.
Defatted whey could also be a source for preparing purified immunoglobulins through the use of UF membranes with large cut-off. However, for this family of milk components, colostrum will probably be a more suitable source than whey.

Glycomacropeptide (GMP) is separated by ultrafiltration with a membrane having a cut-off between 50,000 and 20,000 daltons. GMP suppresses appetite and consequently prevents fatty deposits (results observed with dogs), avoids adhesion of *Escherichia coli* cells to intestine walls, protects against influenza and finally, prevents adhesion of tartar to teeth.

**9. Biological peptides**

Enzymatic modification of milk proteins permits development of peptides having unique physico-functional properties of pharmacological significance. These bio peptides have been implicated in physiological roles such as biotransfer of trace elements, immunomodulation, antihypertension, antithrombosis, regulation of the gastrointestinal tract and the general behaviour (Morphine like activity). UF technology is being used as the most appropriate tool for separating low molecular weight peptides and free amino-acids from proteins substrates utilizing enzymes (Gauthier and Pouliot, 1996; Leppala, A.P., 1996; Maubois *et al.*, 1996).

**10. Lactose**

Another very important dairy ingredient is milk sugar i.e. lactose that has various applications in food and pharmaceutical industries due to its multiple functional properties. Lactose is added to salad dressing, mayonnaise, soups and sauces to enhance flavour and confer added stability to various proteins in the formulations against flocculation at acid pH and pasteurization. The hydrolysis of lactose yields a sweet syrup containing glucose and galactose that have nutritional advantages in some dietary applications. Lactose hydrolysed syrups from permeates and whey's are becoming commercially available and are being used in confectionery and ice cream. A major use of lactose is in humanised infant formulae. Lactose has been used for years in the pharmaceutical industry as a coating agent for pills and tablets. The most recent application for lactose and its derivatives is in the formulation of 'nutraceutical' health foods.

**11. Phospholipids enriched fraction**

Phospholipids enriched fraction can be prepared from whey and buttermilk by using microfiltration. This fraction has valuable emulsifying properties, which could be used by the cheese industry (low fat cheeses), meat industry and cosmetic industry (liposome-based products). Moreover, the nutritional value of the phospholipids will probably lead to further increased interest in this fraction in the next few years.

**12. Dicalcium phosphate**

Whey minerals are the other whey constituents, which can be recovered from the ultrafiltration permeates of acid whey. These melting salts of whey in the form of calcium-magnesium phosphate can be used as food ingredient in meat and fish products (Sienkiewicz and Riedel, 1990).
13. Conclusion

Production of dairy ingredients is a major activity in the developed countries like Europe and United States and provides the Indian Dairy Industry a very exciting opportunity to undertake their manufacture not only to meet the domestic demand but to get into the export markets in United States, Europe and South East Asia. Rapid developments in the range and capabilities of membranes have the potential to profoundly affect the dairy industry as a whole. Being an excellent tool for the fractionation of milk proteins, a new range of products having unique nutritional and functional characteristics (gelling, foaming, emulsification, water holding capacity) have been developed by employing membrane processing. Membrane processing in combination with new efficient demineralization techniques and better knowledge and processes of enzymatic hydrolysis has helped in production of innumerable valuable fractionated dairy ingredients having significantly greater nutritional and biological values that have application in value added products, health foods and pharmaceutical products. More recently, membrane processes have been utilized for the preparation of enzymatic derivatives of milk proteins having pharmacological significance.

14. Selected Reading

Fortification of Milk with Mineral and Vitamins

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Dairy Chemistry Division

1. Introduction

Food fortification may be defined as the addition of one or more essential nutrients to food whether or not it is normally contained in the food, for the purpose of preventing/correcting a demonstrated deficiency of one/more nutrients in the population or specific population groups (Codex Alimentarius Commission, 1994). It is practiced in those areas where the problems of malnutrition are prevalent. This is an effective way to combat the micronutrient deficiency and thus to alleviate “hidden hunger”. Currently, food fortification encompasses a broader concept, and might be done for several reasons. The objectives may be: to maintain the nutritional quality of foods, keeping nutrient levels adequate to correct or prevent specific nutritional deficiencies in the population at large or in groups at risk of certain deficiencies (i.e., the elderly, vegetarians, pregnant women, etc.); to increase the added nutritional value of a product (commercial view); and to provide certain technological functions in food processing. The focus of the international community has so far been on the three most prevalent deficiencies: vitamin A, iodine, calcium and iron.

2. Milk and milk products as a suitable vehicle for fortification

Milk in its natural form is almost unique as a balanced source of man’s dietary need. The various steps in processing and storage have a measurable impact on some specific nutrients. Diet-related micronutrient deficiencies rarely occur in isolation; deficiencies of iodine and vitamin A or of iron and vitamin A or zinc are often observed in the same populations. In addition, widespread deficiencies of some micronutrients, for example, calcium and zinc, may often go undiagnosed because of the absence of specific and sensitive status indicators. Multiple micronutrient supplementations can be more effective in improving nutritional status than supplementation with single key micronutrients; therefore, the multiple fortification of appropriate food vectors, including milk, is of interest from the nutritional standpoint. Milk fortified with multiple micronutrients, chocolate beverages, fruit juices, and soya-based drinks can also serve as excellent carriers.

3. Fortification of milk & milk products with Vitamins

Under ambient conditions the water soluble vitamin C and vitamins of the B-complex group such as thiamin, riboflavin, vitamin B₆, niacin, pantothenic acid, folic acid, biotin and vitamin B₁₂ are powdered and thus relatively easy to work with when producing most dairy products. The fat soluble vitamins which include vitamin A, D, E and K, however, exist either as an oil or as crystals, which may cause processing difficulties during the production of certain types of dairy products (Mortensen and Gotfredson, 1996). One of the problem encountered with the vitamins, is their limited stability in presence of heat, humidity and oxygen. Among the water soluble vitamins, vitamin C, folic acid, vitamin
B₆ and vitamin B₁₂ are the less stable. While in the case of fat soluble vitamins vitamin A, D and E are least stable.

In order to improve the stability of these vitamins, a number of different coating technologies have been developed. One of the most important methods to protect the fat soluble vitamins is microencapsulation, which results in a highly sophisticated powder, where the vitamin is kept protected from degradation by the coating material used for the encapsulation. During microencapsulation, the fat soluble vitamins are brought in the form of oil or a crystal – which in some processes would be difficult to handle – to the form of a free flowing powder much easier to handle and mix with other dry ingredients (Mortensen and Gotfredson, 1996). When two or more vitamins are added to a food product at the same manufacturing stage, this is commonly done in the form of premix or as blend. Premix is a homogenous mixture of desired vitamins in a dry powder form, whereas a blend is the same for the fat soluble vitamins, but in an oily form. A premix can consist of both water soluble and fat soluble vitamins and carotenoids, in which the fat soluble vitamins have to be microencapsulated.

4. Fortification of milk and milk products with iron, calcium and other minerals

Selection of an appropriate mineral fortificant (iron, calcium etc) is based on its organoleptic considerations, bioavailability, cost and safety. The colour of iron compounds is often a critical factor during fortifying milk and milk products. The use of more soluble iron compounds often leads to the development of off-colours and off-flavours due to reactions with other components of the food material. Infant cereals have been found to turn grey or green on addition of ferrous sulphate. Off-flavours can be the result of lipid oxidation catalysed by iron. The iron compounds themselves may contribute to a metallic flavour. Some of these undesirable interactions with the food matrix can be avoided by coating the fortificant with hydrogenated oils or ethyl cellulose (Jackson and Lee, 1991).

Bioavailability of iron compounds is normally stated relative to a ferrous sulphate standard. The highly water soluble iron compounds have superior bioavailability (Richardson, 1990). Bioavailability of the insoluble or very poorly soluble iron compounds can be improved by reducing particle size. Unfortunately this is accompanied by increased reactivity in deteriorative processes. The problem of low bioavailability of some of the less reactive forms of iron is often circumvented by the use of absorption enhancers like, ascorbic acid, sodium acid sulphate and orthophosphoric acid, added along with the fortificant.

The other important mineral for the fortification of milk and milk products, which has been studied, is calcium. Several commercial calcium salts are available for calcium fortification, which include carbonate, phosphate, citrate, lactate and gluconate. In general, organic acid salts of calcium are more bioavailable than inorganic salts (Labin-Godscher and Edelstein, 1996). The pH of the milk should be taken care of during Ca fortification. Manufacturers of calcium fortified milk products should consider adding, magnesium, riboflavin and perhaps vitamin D as well, in amounts that would normally be obtained in a serving of vitamin D fortified milk (Weaver, 1998). Milk and milk products can also be fortified with a range of other mineral salts such as Mg, P, Zn, Cu and Mn. Prudent selection of mineral compounds is based largely on consideration of mineral reactivity and solubility of the salt. To overcome problems of flavour, texture and colour deterioration due to addition of minerals, some companies have engineered new
fortificant preparations, which generally involve the use of stabilisers and emulsifiers to maintain the mineral in solution (FAO, 1995).

5. Technology for fortification

The technology of milk fortification is relatively simple and no additional equipments are needed or can be practiced with minor modifications in the existing plant. Mineral/vitamin fortification can be practiced at several stages in the production. Liquid milk is usually fortified prior to pasteurization or ultra-heat treatment. Homogenization is essential for oily preparations of vitamins. Usually two methods of additions are practiced i.e. batch process for small operations and metered additions for continuous process. A metered injection of the vitamin preparation upstream to the homogenizer has been the standard set up in continuous operation plants (Cornell University, 1999).

Oily preparations are diluted with 10 parts of warm oil (45 – 50°C), usually butter oil and homogenized with a suitable quantity of skim milk or it can be mixed with appropriate quantity of milk and cream and finally homogenized. In the case of water soluble or water dispersible micronutrients, a premix can be made by diluting the nutrients to 20 times their weight with milk at 45°C, followed by stirring and thorough mixing. A simple procedure for fortification of skim milk with vitamin A without using homogenizer was developed by Bector and Rani (1998). This process is basically a batch process and is suitable for small plants of low capital cost.

Many iron compounds have been assessed in the fortification of pasteurized whole milk. The best fortification procedure was judged to be the addition of ferric ammonium citrate followed by pasteurization at 81°C. In this way fortified milk containing 30 ppm iron was found to be acceptable after 7 days storage. Levels of vitamin E, vitamin A and carotene were not affected by the presence of iron. At pasteurization temperatures below 79°C off-flavours developed due to lipolytic rancidity (Edmondson et al., 1971). De-aeration of the milk prior to the addition of iron compounds was also found to reduce flavour problems. In the production of iron fortified evaporated milk, ferric orthophosphate was shown to be useful (FAO, 1995). Calcium fortificant preparations including stabilizers and emulsifiers have been used for fortification of milk and milk-based beverages. It maintains calcium in suspension so as to improve mouth feel and appearance of products (FAO, 1995). In Germany a milk-based fruit beverage has been marketed which is fortified with calcium, phosphorous as well as vitamins A, E, B and C.

6. Conclusion

Fortification ensures a safest method by which manufacturers can deliver health promoting, nutritionally dense food products. It is considered as an emerging technology as it considers the issues of the role of foods in quality of life and the role of foods in reduction of the risk of chronic diseases. The risks associated with fortification are minimal except if good manufacturing practices are not followed and only isolated incidents of this type have ever been reported. Improved understanding of interactions between food ingredients and health and ingenuity of food technologists in food formulation and fabrication will contribute to the advances in food fortification. Many technological problems may occur upon addition of minerals to food products, mainly due to the numerous reactions of minerals with other food components. These problems may be reflected in changes in texture, colour, sedimentation, flavour and/or the functional properties of the product. Prudent selection of mineral compounds is based
largely on consideration of mineral reactivity and solubility of the salt. Analysis of potency of fortificants and of vitamin and mineral content constitute an important component of the overall analytical requirements in QA/QC programmes for fortification processes. Development or selection of appropriate analytical methodologies must be based on consideration of accuracy and precision of measurements, available facilities and equipment, simplicity of procedure and rapidity of determination.

7. Selected Reading


1. Introduction

India has got a pool of medicinal herbs which are proved to be beneficial in curing various diseases. Their regular consumption will not only improve the health status but also reduce risk of lifestyle diseases. However, the direct addition of small amounts (nutritionally not significant) of herbs to a food system, for example, may not adversely affect its organoleptic properties significantly; further incorporating higher levels of the nutrient either to meet certain physiological requirements or to treat an ailment will most often result in unstable and unpalatable foods. Different types of delivery systems have been formulated to overcome these challenges and to provide a wide range of release requirements. A wide variety of different types of delivery systems have been developed to encapsulate functional agents, including simple solutions, association colloids, emulsions, suspensions, gels, solid matrices, etc.

Micro-encapsulation techniques have successfully been employed in the past for the delivery of sensitive bioactive compounds. Among these, emulsion based delivery systems are getting more attention now a days due to their targeted delivery and more absorptive in the human gut. They offer significant advantages over the solids matrix materials as they can be used in liquid food systems. However, stability of these emulsions in the complex food environment has been a challenging work. But many researchers have been successful to improve stability of these emulsions. Some attempts are also made by authors to improve the storage and processing stability of emulsion based delivery systems.

Most of the conventional dairy/food products are prepared using conventional o/w or w/o emulsion technique. With invention of advanced techniques, more complex and novel structures can be created which can successfully deliver these nutraceutical and bioactive ingredients into existing foods. The active ingredients is encapsulated/imbibed in the novel structures like liposomes, vesicles, nano-layers, multiple emulsions, nano-emulsions, multilayer emulsions, solid-lipid particle etc. Thus, technological interventions in emulsion science have opened great opportunities to improve the delivery of bioactive compounds into foods, particularly of probiotics, minerals, vitamins, phytosterols, lutein, fatty acids, lycopene and antioxidants. Several micro-encapsulation technologies have been developed for use in the food industry which demonstrates potentials for the production of functional foods (Table 1.).

2. Emulsion based delivery system

Emulsions are composed of two immiscible phases e.g. oil and water wherein one liquid is dispersed as fine spherical droplets into the other bulk phase. Size of the droplets generally ranges from 100 nm to 100 μm in conventional emulsions, however in nanoemulsions the same is from 20-100 nm. Multiple emulsions are structurally very complex emulsion, where, water droplets are dispersed in larger oil droplet which is further dispersed in the continuous water phase (Garti 1997; Garti and Benichou 2004;
Garti and Bisperink, 1998; McClements, 2010). Multiple emulsions are required for the delivery of sensitive ingredients where direct contact with food matrix adversely affects the performance of bioactive compounds. On the other hand some compounds e.g. Iron, Cu etc are very reactive in free state and brings about undesirable flavours to the finished products. Double emulsions (W/O/W) are most suitable for encapsulation of hydrophilic bioactive components; however they may also be used to deliver both lipophilic and hydrophilic bioactive components in the same system (Cournarie et al., 2004). Their double-compartment structure can be used to encapsulate substances within the internal water droplets or to reduce the fat content of emulsion-based products, (McClements et al., 1999). If these compounds are encapsulated using multiple emulsion technique, this could lead to development of novel products. Thus, multiples emulsions offer more scope for the control of encapsulation ingredient, protection and release mechanism as compared to conventional emulsions.

**Table 1. Bioactive ingredients need to be delivered in foods**

<table>
<thead>
<tr>
<th>Name</th>
<th>Types</th>
<th>Potential health benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acids</td>
<td>ω-3 fatty acids, conjugated linoleic acid,</td>
<td>Coronary heart disease, bone health, immune response disorders, weight gain, stroke</td>
</tr>
<tr>
<td></td>
<td>butyric acid</td>
<td>prevention, mental health, cancer, and visual acuity</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>β- Carotene, lycopene, lutein, and zeaxanthin</td>
<td>Cancer, coronary heart disease, macular degeneration, and cataracts</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>Tocopherols, flavonoids, polyphenols</td>
<td>Coronary heart disease, cancer, and urinary tract disease</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>Stigmasterol, β-sitosterol, and campesterol</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>Proteins, Peptides,</td>
<td>Caseins, ACE, lactoferrin, lactoperoxidase,</td>
<td>Brain functioning, bioavailability of minerals, cancer, bone health, immuno-modulation,</td>
</tr>
<tr>
<td>Amino acids</td>
<td>immunoglobulin</td>
<td>hypertension</td>
</tr>
<tr>
<td>Bioactive carbohydrates</td>
<td>Dietary fibers, prebiotics e.g. GOS</td>
<td>Coronary heart disease, cancer, diabetes, constipation</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Probiotics,</td>
<td>Intestinal health, cancer, immunity</td>
</tr>
<tr>
<td>Essential minerals</td>
<td>Iron, zinc, calcium, selenium, chromium</td>
<td>Anaemia, growth and development, bone health, diabetes, cancer and thyroidism</td>
</tr>
<tr>
<td>Herbs</td>
<td>Puereia tuberosa, Bacopa monniera, Withania somnifera, Asparagus racemosus</td>
<td>Medicinal properties</td>
</tr>
</tbody>
</table>

2.1 Structural design of emulsions based delivery systems

Selection of structural components in emulsion based delivery systems depends upon target applications, final continuous phase and characteristics of bio-active ingredients. The building blocks of a delivery system consist of three constituents, i.e. lipid phase, surfactant and biopolymer. A variety of materials (Table 2.) are being used currently to develop emulsion based delivery systems. Wide range of materials both for cores and wall and also various technologies, for controlling the interactions of ingredients in a given food system and, for manufacturing microcapsules and microparticles of different size, shape, and morphological properties are being evaluated.
Table 2. Major food-grade structural components used to construct delivery systems for bioactive components.

<table>
<thead>
<tr>
<th>Name</th>
<th>Important characteristics</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids</td>
<td>Non polarity</td>
<td>Animal fats: beef, pork, chicken</td>
</tr>
<tr>
<td></td>
<td>Chemical stability</td>
<td>Fish oils: cod liver, menheden, salmon, tuna</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plant oils: palm, coconut, sunflower, safflower, corn, flaxseed, soybean</td>
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<tr>
<td></td>
<td></td>
<td>Flavor oils: lemon, orange</td>
</tr>
<tr>
<td>Surfactants</td>
<td>Solubility (HLB)</td>
<td>Non-ionic: Tween, Span</td>
</tr>
<tr>
<td></td>
<td>Head group charge</td>
<td>Anionic: SLS, DATEM, CITREM</td>
</tr>
<tr>
<td></td>
<td>Molecular geometry</td>
<td>Cationic: lauric arginate</td>
</tr>
<tr>
<td></td>
<td>Surface load at saturation</td>
<td>Zwitterionic: lecithin</td>
</tr>
<tr>
<td>Biopolymers</td>
<td>Molar mass</td>
<td>Globular proteins: whey, soy, egg</td>
</tr>
<tr>
<td></td>
<td>Conformations charge</td>
<td>Flexible proteins: casein, gelatin</td>
</tr>
<tr>
<td></td>
<td>Hydrophobicity</td>
<td>Nonionic polysaccharides: starch, dextran, agar, galactomannans, cellulose</td>
</tr>
<tr>
<td></td>
<td>Flexibility</td>
<td>Hydrophobicity Anionic polysaccharides: alginate, pectin, xanthan, carrageenan, gellan, gum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arabic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cationic polysaccharides: Chitosan</td>
</tr>
</tbody>
</table>

2.2 Double emulsions

(i) Water in oil in water (w/o/w): Double emulsions are compartmentalised liquid dispersions in which the droplets of the dispersed phase contain smaller dispersed droplets that are similar to the continuous phase. Water-in-oil-in-water (W1/O/W2) double emulsions contain three distinct phases; an inner aqueous phase (W1), which is encapsulated in an oil phase (O), which is enclosed within a second aqueous phase (W2) (Fig.1).

(ii) Oil in water oil (o/w/o): Oil in water in oil double emulsion consists of three distinct phases; an inner oil phase, which is encapsulated in an aqueous phase, which is enclosed within second oil phase.

Application:-

- to encapsulate lipophilic bioactive components are β-carotene and ω-3 fatty acids
- to encapsulate hydrophilic bioactive components such as vitaminB, immunoglobulins insulin, proteins, and amino acids
- to prepare a delivery system that contains both lipophilic and hydrophilic bioactive components in the same system

Advantage:-

Potentially, W/O/W emulsions have some advantages over conventional O/W emulsions as delivery systems for bioactive lipids, although they are normally more suitable for encapsulation, protection, and release of hydrophilic components.
Functional components could be trapped inside the inner water droplets and released at a controlled rate or in response to specific environmental stimuli, for example, in the mouth, stomach, or small intestine.

Active ingredients could be protected from chemical degradation by isolating them from other water-soluble ingredients that they might normally react with.

This techniques could utilized to produce reduced fat products with similar physicochemical and sensory properties as full-fat products, for example, appearance, texture, mouthfeel, and flavor.

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**Fig. 2. Schematic diagram of double emulsion w/o/w preparation**
(Source: Okochi & Nakano, 2000)

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### 2.3 Encapsulation efficiency and encapsulation stability of double emulsions

Encapsulation efficiency (EE) of a delivery system is defined as the quantity of the internal aqueous phase ($W_1$) which remains intact in the double emulsion ($W_1/O/W_2$). EE in the multiple emulsions can be determined by incorporating known quantity of marker in the internal phase and comparing it with the leached amount of marker to the outer aqueous phase. More the losses of the marker, less is the EE of the system. Various markers have been tried in the past i.e. methylene blue, vitamin B$_{12}$, NaCl etc. Whereas encapsulation stability is the difference in the EE of the fresh sample and stored or environmentally stressed samples. Thus, ES is the ability of double emulsions to resist the environmental changes such as temperature, gravitational force, centrifugal force etc. Most widely used method to determine these properties is centrifugal method wherein the prepared fresh double emulsion having marker in the inner phase is subjected to force to separate cream phase and outer aqueous phase. The various methods are used to quantify
the marker viz. absorbance, transmittance and conductivity measurement, titrations etc (O’Regan and Mulvihill, 2009; Lutz et al. 2009 and Sapei et al., 2012). Sedimentation stability is another way to express the structural rigidity of the multiple emulsions which is defined as the ratio of the sedimentation height to the initial height of the emulsion placed in the measuring cylinder or tubes (Sapei et al., 2012). It is also expressed as percent stability.

2.4 Microfluidization as a method for the emulsion based delivery systems

Recently, many attempts have been made to generate emulsion droplets in microfluidic devices. Single emulsions, which involve a process for breaking two immiscible fluids into smaller droplets, were extensively investigated in recent years using these devices. In this system the pressurized product stream (Fig. 2) enters the interaction chamber and passes through geometrically fixed micro channels, causing it to accelerate to very high velocities. It is here that the product stream is acted upon by two primary forces, which bring about the desired results. It creates shear - deformation of the product stream; occurring from contact with channel walls at high velocity and also the impact - collision; occurring when the high velocity product stream impinges upon itself. Both these phenomenon are responsible for the size reduction to the nano-scale.

![Fig. 2. Schematic of the Microfluidizer processor](image)

3. Conclusion

Micro-encapsulation techniques have successfully been employed in the past for the delivery of sensitive bioactive compounds. Among these, emulsion based delivery systems particularly multiple emulsions (e.g. W/O/W) are getting more attention nowadays due to their targeted delivery. They offer significant advantages over the solids matrix materials as they can be used in liquid food systems. However, stability of emulsions in the complex food environment has been a challenging work. But many researchers have been successful to improve stability of these emulsions. More research is required in the area of delivery systems, especially for the liquid products such as milk and milk based drinks.

4. Suggested Reading


1. Introduction

Human infants should ideally be nursed on mothers milk which constitutes natures best food. However, in the event of lactation failure, insufficient milk secretion and where mothers suffering from transmittable diseases, human milk substitutes serve as savers of precious life during vulnerable stages of infancy. Most of the breast milk substitutes utilize bovine milk due to easy availability. There has been an ever increasing reliance of formula feeding practices both in developed and developing countries. There has been an tremendous growth in the dried milk industry for manufacture of infant formula. Today nearly 185,000 tonnes of infant formula is manufactured in India representing approximately 3.8% of total milk production.

2. Standards of infant formula

During 1960s the knowledge regarding various biochemical differences and resultant physiological stress of formula feeding was limited and therefore, earlier standards (IS: 1547:1960) mainly paid attention to the adjustment of fat content. With the view to provide nutritionally improved formulas these standards were brought to the conformity with those of Codex Alementerious Commission with adoption of WHO code for promotion of proper infant feeding practices (IS: 11156:1985). Emphasis has been laid on manufacture of formulations having compositional and biochemical characteristics similar to human milk. The changes proposed in the new standard envisaged closer proximity to human milk and minimum physiological stress due to adaption. Provisions have been made to incorporate 12% PUFA rich vegetable oils in order to simulate fatty acid profile of the formulation with respect to ratios of small : medium: long chain fatty acids, unsaturated: polyunsaturated fatty acids, and linoleates similar to human milk. The protein content has been lowered owing to the physiological stress on immature kidney during infancy. However, adequate supply of essential amino acids and their optimal levels for efficient utilization has been taken care of. The Minstry of Health and Family Welfare through a Gazette notification has amended the Prevention of Food Adulteration rules (5th Amendment,1991-92) in respects to the industrially prepared infant formulations also a number of new formulations have been introduced specifically to cater the special needs of infants born with specific physiological disorders.

3. Developments in infant formulation

It is often noticed that as the infant grow, mother milk does not suffice his growth requirements and therefore, cow or buffalo milk, depending upon availability, was invariably diluted with water and fed to the infants. Dried milk formulations for infant feeding have been developed on the same lines. In view of easy availability most breast milk substitutes utilize bovine milk for industrial preparations, however, it is important to recognize that various mammalian offsprings are born at different stages of intrauterine development and therefore undergo different growth rates. The milk composition of various species therefore, differs so as to meet the nutritional requirement of their offsprings. The relative ratios of protein: fat: carbohydrates: minerals as well as makeup
of these nutritionally important constituents differ appreciably between human and bovine milks.

3.1 Standard formulas

These formulations are based on bovine milk and work as supplement or replacement of human milk for feeding of normal healthy infants. The nutritional requirements of infants through such formulations are met mainly by course adjustments of Protein: Fat: Carbohydrates ratios as a broad compositional simulation to human milk. These formulations are also fortified with general minerals and vitamins. They are generally referred to as first generation formulas developed to meet the general energy and nutritional requirements of infants born normally with out any physiological disorders.

3.2 Soy based formulas

Certain infants are born with certain physiological disorders of transitional nature. Depending upon the nature of disorder their requirements for nutritional and normal growth changes. Under the circumstances standard formulations may not be suitable for such infant. Infant formulas based on soy proteins have been developed to aid in the dietary management of infants suspected of being allergic to milk protein. The protein content of such formulations are replaced either from soy flour or water soluble soy protein isolates. Other vegetable protein, with or without suitable modifications, can also be used for the formulation of such products. Being devoid of major milk sugar, Lactose these formulations can also be used for the dietary management of infants suffering from lactase deficiency.

3.3 Specialised formulas

This group of formulas are specific products for the dietary management of infants with variety of nutritional and physiological disorders that are associated with the early stages of life. Certain infants, for example, are born with disorders of enzymic insufficiency to digest milk proteins or carbohydrates present in standard formulation. Such infants require protein to be present on the formulation in pre-digested from that is easy to digest or hydrolyzed protein, hydrolyzed lactose, low fat formula etc. fall into this category. These formulations are designed to have minimum load on the under-developed digestive system. Such formulations are sold under specific brand names depending upon their applications.

3.4 Nutritionally improved formulas

Currently marketed infant formulas differ considerably from human milk in compositional and biochemical characteristics and thus display several nutritional inadequacies. Feeding of such formulas have proved to cause different physiological stress amongst infants. Nutritionally superior formulations have been developed for easy adaptability. These formulations bear varying degree of chemical and biochemical similarity with human milk and are refered to as humanized or maternalised formulas. These humanized formulas posses similar ratios of major nutrients with regard to protein: fat: carbohydrate : minerals found in human milk. Apart from similar gross compositional profile these formulation also incorporate comparable ratios of whey protein: Casein; saturated : polyunsaturated fatty acid; calcium : phosphorus and sodium : potassium. Such formulations closely approach the nutritional qualities of human milk and therefore are easily adaptable by the infantile system without causing great physiological stress on the under-developed organs.
4. Adjustment of fatty acid composition

The ratio of unsaturated to saturated fatty acids in infant formulas has been adjusted to that of human milk by incorporation of soybean, oleo and safflower oils which are rich in unsaturated fatty acids and coconut oil and milk fat, which are rich in saturated fatty acids. Corn oil may also be added to formula to increase the oleic acid content. Since cow and goat milk fat also contain highly absorbable triacylglycerols with palmitic acid in the Sn-2 position, the digestibility of infant formulas could be improved by blending vegetable fats and milk fats. Such formulations have ratios of saturated : polyunsaturated fatty acid, short : medium : long chain fatty acids and Linoleic acid content similar to human milk.

5. Modification of milk proteins

Hypersensitivity to cow milk has caused a significant health problem since the introduction of mass-produced infant formulas at the beginning of the twentieth century. Allergic symptoms normally appear within the first two months of life and have been noted in 1 to 8 percent of formula fed infants. Although the β-lactoglobulin fraction has been implicated most often in allergic reactions to cow milk, the caseins, α-lactalbumin, serum albumin and immunoglobulins and digests of these proteins are also allergenic in infants and children. The β-lactoglobulin can be removed and the remainder of the proteins blended for use in infant formulas. The caseins of cow milk also have been hydrolysed and subjected to gel filtration to prepare a non-antigenic fraction. Kuchroo and Ganguli (1980) prepared a humanized infant formula from buffalo milk containing low levels of the antigenic αs casein. Electrodialysed skim milk was subjected to protein hydrolysis with trypsin, pasteurized and fortified with lactose and vegetable oils. After homogenization, vitamin mix was added and mixture spray dried. Commercial hypoallergenic infant formulas such as Neutramagin and Pregestamil form Mead-Johnson contain casein hydrolyzates.

6. Modification of carbohydrates profile

Having adjusted protein and fat requirements, caloric density of the formulation was adjusted through the incorporation of carbohydrates. After accounting for the energy available from protein and fat, the carbohydrates requirement was calculated to be 61.10%. The bovine milk used in the formulation (mainly as a source of proteins) accounts for only 18.9% of lactose. Balance amount for the total carbohydrate is derived from the exogenous sources, namely maltodextrins and sucroses. The relative ratios of maltodextrins and sucrose to control the level of sweetness in IF is based on. It appears that at 4.55% level, sucrose masks adequately extraneous flavour in LIF that may originate from vegetable oils used for formulation. Thus maltodextrin and sucrose in the ratio of 37.7:4.5 may be recommended. The total carbohydrates content of 61.6 %(W/W) would contribute 51% of the total calories available from the feed. In normal human milk, lactose contributes about 41% of the total calories. Disorders in lactose digestion among infants calls for reduction in lactose content of feeds to the ‘tolerance levels’. Reduction of lactose by 30% through enzyme treatment would result in a lactose content of 0.89% in IF which is within the normal limits of ‘tolerance’ (Barness 1981).). Secondary forms of lactose intolerance are associated with atrophy of the small intestinal epithelium or complete atrophy of the small intestinal epithelium or complete atrophy of the villi, due to enteric infections or allergy to dietary proteins. In such events, lactase activity often remains very low, whereas the other disaccharides, viz. maltase and sucrase may be at normal levels, specially during the recovery period. ‘Sweetness’ in liquid feeds is associated with increased intake of formulas and obesity among infants (Jaguier 1981). It is for this reason that liquid feds for infants must have neutral taste to permit self
regulation of feed intake. Thus maltodextrin and sucrose would be suitable source of energy in the dietary management of lactose intolerant infants.

7. Modification of mineral profile

In view of the major differences between in buffalo milk and human milk, with regards to the total mineral content and make up of various nutritionally significant minerals, attempts were made to modify the ash content, Ca:P ratio, Na:K ratio and Fe content to suit the nutritional and physiological requirements of infants was attempted.

7.1 Ash content

In contrast to human milk, buffalo skim milk contains almost 3 times higher ash content. Dried buffalo skim milk contains about 7.38 percent total ash which is adjusted to 2.65 percent in IF. The ash content in IF can be lowered through extraneous incorporation of fat, maltodextrin and sucrose. Lower ash content is specially desirable in lowering the osmolar load on the undeveloped kidney among infants.

7.2 Ca:P ratio

With the objective to enhance utilization of dietary Ca and consequently fat absorption amongst infant, the ratio of Ca:P of IF need to be adjusted so as to simulate human milk. In case of human milk, Ca:P ratio is about 2:1 while it is 1.61:1 in case of buffalo milk. With unmodified IF, about 0.79g of Ca and 0.49 g of P per 100g of spray dried product were observed. Therefore,2.11 kg of pharmaceutical grade of calcium gluconate (that would contribute 18 g of Ca is to be added per 100 kg of dried LIF to simulate human milk. AAP (1976) recommended that Ca:P ratio in any infant formula should not be less than 1.1:1 and should not be more than 2:1 for better absorption of Calcium.

7.3 Na: K ratio

With a view to maintain extracellular and intracellular concentration of water at normal levels, Na:K ratio is modified in IF to stimulate human milk. The Na: K ratio of human milk is about 1:3.67 which is much lower than that of buffalo skim milk (1:2.18). With unmodified Na:K ratio 0.24g of Na and 0.53g K per 100g of LIF were observed. 782g of potassium chloride (an approved additive to the infant formula by Codex Alimentarius Commission (1976) was therefore, to be added in 100kg to modify Na:K ratio of LIF.

In contrast to sodium, which is known to be associated with blood pressure, potassium has reciprocal relationship with sodium in many biological functions, including lowering blood pressures in infant’s diet by increasing potassium would help normalize blood pressure (Lawerence 1980).

7.4 Iron (Fe) content

Lack of Fe in infant feed often lead to iron-deficiency anaemia both in normal and lactase deficient infants. Since buffalo milk contains only trace amounts of Fe 11.61g of ferrous sulphate (Pharmaceutical grade) was added to provide 5mg of Fe per 100g of LIF as recommended by Codex Alimentarius Commission (1976).

8. Human milk based formula

A blend with whole human milk, human cream, salt and lactose free human milk protein powder into a high protein, high fat and high protein,high fat and high-energy infant formula for birth-weight infants was prepared in the 1980s. The increased concentration of antimicrobial protein allowed subsequent pasteurization of the formula with minimal loss in antimicrobial activity.
8.1 Addition of lysozyme

Egg white lysozyme has a similar composition, though different antigenic specificity, than human milk lysozme, and is readily available for use in infant formulas. Humanized infant formulas containing egg white lysozme have been patented by Japanese Corporation. Russian workers developed and patented procedures for supplementing fermented infant formulas with lysozmes in order to increase their biological value. The administration of lysozme-supplemented preparation in addition to other therapeutic agents, decreased the time required to treat Russian children hospitalized for anemia and pneumonia. Although it has been reported that infants given lysozme treated formula have increased intestinal levels of bifidobacteria, lysozmes itself does not appears to stimulate the growth of bifidobacteria in cow milk.

8.2 Addition of bifidobacteria

Notwithstanding the nutritional superiority, the Humanized formulations do not offer bioprotective attributes of ready to use dried infant formula, a process was developed to incorporate a biomass of Bifidobacteria for its protective role in gut ecology. Being native to human gut ecological system, it has capabilities for implantation at mucosal layer and control the growth of other intestinal bacteria that results in maintaining the balance of intestinal microflora and thereby control enteric infections. The use of whey for adjustment of whey protein: casein ratio in the formula, enhances Bifidus growth stimulating activity. Such formulations are helpful in implanting Bifidus microflora in the intestinal tract of infants through bottle feeding for quick restoration to normalcy during diarrhoeal attacks. With this advancement infant formula has come a step closer in fulfilling one of the biological functions unique to human milk for infant nutrition. The preparation of bifidobacterial starters and their use in fermented infant formulas has been described and patented. Studies at the National Dairy Research Institute in Karnal, India compared the effects of feeding a non-fermented infant formula fortified with a spray dried concentrate of bifidobacteria. Fortification decreased the fecal pH and increased the bifidobacteria to the level obtained with human milk. The fecal coliform counts decreased from 7.50 to 7.09 cfu/ml as compared to 6.6 for human milk. The retention of calcium, phosphorus and iron was also significantly increased, mostly probably because of the low intestinal pH. With the exception of a carbohydrate preparation and “bifogenic factor” described by Russian workers, the incorporation of bifidus stimulating factors other than lactulose into prepared by heating lactose and incorporated into formulas to increase the intestinal population of bifidobacteria in infants. When lactulose was administered to infants simultaneously with a freeze-dried preparation of bifidobacteria there was a greater reduction in intestinal pathogenic E.coli than when culture alone was given.

8.3 Addition of other lactobacilli

A number of other closely related lactobacilli, which inhabit the human intestinal tract synthesis organic acids (acetic, benzoic, lactic ), hydrogen peroxide, B-vitamins and enzymes which may provide nutritional and/ or therapeutic benefits. The lactobacilli also produce natural antibiotics with broad-spectrum activity against pathogenic and non-pathogenic organism. For example, acidophilin, acidolin and lactocidin are produced by L.acidophilus, lactobacillin (H₂O₂) by L.brevis, bulgarin by L.bulgaricus and lactolin by L.plantarum. Milk fermented with L.acidophilus, or a mixed culture of L.acidophilus and B.bifidus have been used successfully in the treatment of enterocolitis and other intestinal disorders in adults. Dietary supplements of L.acidophilus and B.bifidium tablets or acidophilus milk have been used to adjust the intestinal flora of newborn premature and full-term infants, respectively.
Acidophilus milk was more effective than antibiotic treatment in controlling E. coli mediated infantile diarrhea and Shigella and salmonella mediated-dysentery in children in Poland. Yakult, a Japanese fermented milk product containing L.acidophilus Shirota, has been used extensively for the treatment of intestinal disorders in infants and children. Infants treated with yoghurt (prepared from a mixed culture of Streptococcus thermophilus and L.bulgaricus) showed a more rapid recovery from diarrhea than those given a Neomycin-kapectate mixture. The administration of yoghurt cultures has also been shown to increase the proportion of bifidobacteria in the infant intestine. Acidified infant formulas and those fermented with a S.lactis have been found to protect infants against salmonella infection.

8.4 Lactoferrin

Lactoferrin is capable of chelating iron thus depriving bacteria of the iron required for its normal metabolism resulting in their inhibition. Digestion of lactoferrin with proteolytic enzymes does not significantly alter its iron binding property, suggesting its quite capable of exerting antibacterial activity in human intestinal tract. The biological role postulated for lactoferrin includes bacteriostatic and bactericidal activity, an enhancer of monocytes natural killer activity and modulator of activation of complement system. Lactoferrin is also found to be involved in the transport of iron across the duodenal brush border. Thus incorporation of lactoferrin in infant formula can help in bioavailability of iron and preventing growth of gram negative pathogenic bacteria. Lactoferrin may be extracted from whey, a byproduct of cheese manufacture, in its native from that holds promise for incorporation of this antibacterial protein of infant formulas of the future.

8.5 Lactoperoxidase

In the new born infant saliva is quite rich in lactoperoxidase activity and the enzyme is known to be resistant to low pH and proteolysis. The saliva that enters the stomach and rich in lactoperoxidase is expected to reach the intestinal tract in an active form for activation of LP system. Controlled application of the system in infant formula may be very useful for protecting neonatal health.

8.6 Lysozme

In view of the antibacterial effect and possibly general immune system of neonate, there has been a considerable interest in developing food applications of lysozyme. The concentration of lysozme in human milk is 3000 times greater than bovine milk. The biological significance of lysozyme lies with its possible protective effect against gram negative potential pathogen. Although lysozme is isolated from egg, bovine lysozyme has greater lytic activity. It is stable at low pH and resist gastric juices under in-vivo situations. Lysozyme containing formulations tend to enhance Bifidobacterium population in the intestinal tract. Thus the incorporation of lysozyme in infant formula could be useful in view of its influence under the ecological system of the intestinal tract.

8.7 Immunoglobulins

Intestinal immunity is the result of highly complex interaction of humoral and cellular components. The bactericidal properties of milk are attributed to the complement mediated antibody system. The secretory IgA which displays unique property for the defence of mucosal membrane is inadequate during infancy as its is not reabsorbed among infants. Bovine milk which ids deficient in IgA, however, provides strong bactericidal and bacteriostatic effects involving IgM and IgM. The post colostral milk displays varying levels of bactericidal activity against E.coli. This could potentially influence the gut microflora and perhaps responsible for gradual suppression of E.coli.
Ingestion of heated milk produce circulatory antibodies to beta-lactoglobulin and casein which seems to be critical in the retention of antigenic properties of milk. The bovine colostrums provides neonatal ingulates a rich supplement of antibodies. Possibilities thus, exist to incorporate lipohysed bovine collostral whey into the infant formula.

9. Packaging

It is an important aspect that is essential for safe and easy marketing of any food item and retention of natural characteristics. A wide variety of rigid as well as flexible packaging materials are available and used for packaging of food products World wide. Generally infants foods are packed in metalised tin containers which can be sealed, oxygen evacuated and filled with an inert gas to protect the contents against chemical and microbiological spoilage. Tin containers having snap on plastic lids are now available that can keep the contents safe against moisture, air and pests. In order to reduce the ever increasing cost of tin containers, laminated flexible packaging material are in great use. The laminated pouches, after packaging and sealing are then placed into individual cardboard carton for easy handling. Processes are also available where inert gas can be injected in these laminated packages to avoid chemical deterioration (oxidation) of susceptible constituents during storage at ambient temperature. The western world, liquid infant formulations are available in aseptically packed simple glass bottles or food grade poly bottles. These are used as single service throw away containers. The content of each bottle is sufficient for one time feed requirements of an infant.

10. Conclusion

The technological advancement for the production of infant formula has gone a long way in manufacture of variety of infant formulas for dietary management of normal, preterm-infants, and infants born with variety of nutritional as well as physiological disorders. Both nutritionists and biochemists have provided ample knowledge for the technocrats to manufacture infant formulas having similarities in general characteristics to the bioprotective attributes of infant formulae that could provide humoral protection in the infantile gut ecological system. These factors are vital for establishing a healthy microflora in the intestinal tract and potentially play an important role of protecting the infant against enteric infections.

11. Selected Reading


1. Introduction

Metabolism is the sum of the chemical processes that take place in the cells and fluids of the body that converts food into energy. This includes the transportation and absorption of nutrients and minerals, the breakdown and buildup of large molecules, the interconversion of small molecules, and the ultimate production of energy from these chemical reactions. As every chemical step of metabolism is catalyzed by an enzyme, a major part of a healthy metabolism is the generation of enzymes which break food down into energy and handle the transport of that energy. If a genetic abnormality affects the function of an enzyme or causes it to be deficient or missing altogether, various disorders can occur that are known as metabolic disorders. This results in inability to break down some substance that should be broken down, leading to intermediate substance that is often toxic to build up, or from an inability to produce some essential substance. In the later case, the host organism fails to get proper nutrition, even if it is eating a healthy, balanced diet. A metabolic disorder can cause a wide range of symptoms including muscle weakness, neurological problems, intestinal irregularities, and cardiovascular problems, among many others. The World Health Organization (WHO) criteria of metabolic syndrome i.e., the symptoms caused by the malfunctioning metabolic processes are:

1. Fasting plasma glucose: $\geq 100$ mg/dl
2. Impaired plasma glucose tolerance: $\geq 140$ mg/dl, two hours after 75g glucose challenge. Plus, any two of the following
   1. Dyslipidaemia: Plasma triglycerides $\geq 150$ mg/dL
   2. Dyslipidaemia: High density lipoprotein (HDL) cholesterol $<35$ mg/dL (men) or $<39$ mg/dL (women)
   3. Hypertension: ($\geq 140$ mm Hg systolic or $\geq 90$ mm Hg diastolic) or taking blood pressure medication
   4. Adiposity: Body Mass Index (BMI) greater than 30 and/or waist:hip ratio $>0.9$ in men, $>0.85$ in women
   5. Microalbuminuria: Urinary albumin excretion rate $\geq 20$ µg/min or albumin:creatinine ratio $\geq 30$ mg/g

Since the clinical symptoms can be vague, diagnosis is complicated, especially in regions where people do not have access to excellent health care. This article focuses on some key requirements of dairy foods that are suitable for the people are suffering or prone to metabolic disorders.

2. Dairy foods for lowering blood glucose level

The effect of foods on blood sugar level is measured by Glycemic Index (GI). Foods that contain types of carbohydrates that break down quickly during digestion and release glucose rapidly into the bloodstream have a high GI, whereas carbohydrates that break
down more slowly, releasing glucose more gradually into the bloodstream possesses a low GI. A lower glycemic index suggests slower rates of digestion and absorption of the food carbohydrates and indicates greater extraction of the products of carbohydrate digestion from the liver. A lower glycemic response usually relates to a lower insulin demand and helps long-term control of blood glucose as well as blood lipids. The glycemic index of foods depends on a number of factors such as the type of starch (amylose versus amylopectin), physical entrapment of the starch molecules within the food, fat and protein content of the food and organic acids or their salts in the food. High, medium and low glycemic index foods have GI values > 70, between 56-69 and < 55, respectively. Table I shows the glycemic index of some common foods. Most of the dairy products are naturally low in GI, however fortifying them with soluble fibers e.g., psyllium, pectin, guar gum, fructo-oligosaccharides etc. lower GI even further by speeding up the gastric emptying rate.

Milk proteins have received increased attention as potential ingredients of health-promoting functional foods targeted at diet-related chronic diseases, such as cardiovascular disease, type 2 diabetes and obesity. Among these, physiologically active milk peptides are under intense consumer and industry interest. These peptides are in a latent or inactive state within protein molecules, but can be released during enzymatic digestion in gastro-intestinal tract, fermentation of milk with proteolytic starter cultures or hydrolysis by proteolytic enzymes (Ghosh, 2011). Natural dietary bioactive peptides which can induce satiety and reduce insulin resistance that helps in combating type 2 diabetes.

3. Dairy foods for lowering dyslipidemia

Due to the association with high dietary cholesterol and saturated fat dairy foods have been implicated to contribute to development of dyslipidemia, one of the most potential risk factors for cardiovascular diseases. It has been reported that only one percent increase in energy as saturated fatty acids would elevate blood cholesterol by 2 mg/dl. However, contrary to the traditional belief, recent findings suggest that dietary patterns with high dairy product intake are associated with reduced risk of the components of metabolic syndrome. Some studies have also demonstrated direct negative association of dairy food consumption and components of metabolic syndrome, e.g., high blood pressure and adiposity, which are also risk factors for type 2 diabetes. There are basically two approaches for development of dairy products to fight dyslipidemia:

3.1. Low-fat dairy products

A DASH (Dietary Approaches to Stop Hypertension) study conducted in USA evaluated the effects of a healthy diet that included low-fat dairy products (milk, yogurt, and cheese), fruits, and vegetables on blood pressure in ~450 subjects for eight weeks. Results showed that, prepared by combining low-fat milk, cheese or yoghurt with fruits and vegetables “the DASH diet,” resulted in the greatest reductions in blood pressure compared to a fruit and vegetable diet that excluded the low-fat dairy products was about half as effective as the DASH diet. Another study examined the effects of the DASH diet in subjects with metabolic syndrome. Compared with the control diet, the DASH diet led to increased HDL, lower triglycerides, lower blood pressure, weight loss, and reduced fasting blood glucose in both men and women.
Table 1. Glycemic Index of common foods

<table>
<thead>
<tr>
<th>Dairy Products</th>
<th>Snacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk (whole) 22</td>
<td>Cashews 22</td>
</tr>
<tr>
<td>Milk (skimmed) 32</td>
<td>Peanuts 14</td>
</tr>
<tr>
<td>Milk (chocolate flavored) 34</td>
<td>Popcorn 55</td>
</tr>
<tr>
<td>Ice Cream (full-fat) 61</td>
<td>Potato Chips 55</td>
</tr>
<tr>
<td>Ice cream (low-fat) 50</td>
<td>Noodles (instant) 46</td>
</tr>
<tr>
<td>Yogurt (low-fat) 33</td>
<td>Walnuts 15</td>
</tr>
<tr>
<td>Cheese 0</td>
<td>Sugars</td>
</tr>
<tr>
<td>Paneer 0</td>
<td>Fructose 23</td>
</tr>
<tr>
<td>Grains</td>
<td>Glucose 100</td>
</tr>
<tr>
<td>Basmati Rice 58</td>
<td>Honey 58</td>
</tr>
<tr>
<td>Brown Rice 55</td>
<td>Lactose 46</td>
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<tr>
<td>Fruits</td>
<td>Maltose 105</td>
</tr>
<tr>
<td>Apple 38</td>
<td>Sucrose 65</td>
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<tr>
<td>Banana 55</td>
<td>Vegetables</td>
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<tr>
<td>Grapes 46</td>
<td>Beets 69</td>
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<tr>
<td>Mango 55</td>
<td>Cabbage 10</td>
</tr>
<tr>
<td>Orange 44</td>
<td>Carrots 49</td>
</tr>
<tr>
<td>Papaya 58</td>
<td>Corn 55</td>
</tr>
<tr>
<td>Pear 38</td>
<td>Green Peas 48</td>
</tr>
<tr>
<td>Pineapple 66</td>
<td>Mushrooms 10</td>
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<tr>
<td>Watermelon 103</td>
<td>Potato (baked) 93</td>
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<tr>
<td>Breads</td>
<td>Sweet Potato 54</td>
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<tr>
<td>White Bread 70</td>
<td>Pumpkin 75</td>
</tr>
<tr>
<td>Whole Wheat Bread 69</td>
<td>Onion 10</td>
</tr>
</tbody>
</table>

3.2. Fortification with specified nutraceuticals

Dietary fibers have long been acknowledged as food components that are beneficial to health. Dietary fibers can be either soluble or insoluble in water. Soluble dietary fibers have been associated with reducing the blood cholesterol levels. The body uses cholesterol in the production of bile acids some of which are excreted daily. The consumption of water-soluble fiber binds to bile acids, which result in an increased excretion of cholesterol. A number of soluble fibers, e.g., pectin, β-glucans, polydextrose, hydrolyzed guar gum etc. could be used in dairy-based beverages.

Insoluble fibers increase bulk and lowers the speed of passage of foods through the GI tract, reduces the risk of colon cancer and diverticulitis (formation of small pouches in the colon). By selecting the appropriate one, insoluble fibers (like lignin, cellulose, hemicellulose, resistant starches) could also be used effectively in dairy beverage products. Particle suspension using fluid gel technology/microfluidization could also be used for incorporating insoluble fibers in milk beverages.

Omega-3 fatty acids are polyunsaturated fatty acids essential to human health but cannot be manufactured by the body, hence must be obtained from food. Omega-3 fatty acids can be found in fish and certain plant oils. There are three major types of omega 3 fatty acids that are ingested in foods and used by the body: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Extensive research
indicates that omega-3 fatty acids help to prevent certain chronic diseases such as heart disease, arthritis, foster brain and visual development and improves immune reaction against allergies. A number of dairy beverages fortified with omega-3 fatty acids are already available in the supermarket shelves in US, Canada, Europe and Australia. Fermented milk products and cheeses made with probiotic cultures could also be fortified with plant sterols and stanol esters. The later ingredients have been authorized by the US Food & Drug Administration (FDA) for the use of labeling health claims for their ability to reduce the risk of coronary heart disease (CHD) by lowering blood cholesterol levels.

4. Dairy foods for lowering hypertension

Several bioactive ingredients derived from fractionation of milk protein could play an important role in reducing hypertension. Hydrolysates of whole milk proteins both caseins and whey proteins are good source of casokinin and lactokinin. Casokinin and lactokinin are Angiotensin-I Converting Enzyme Inhibitory Peptides (ACE-IP). Inhibition of ACE results in lowering blood pressure and hence, fermented dairy products or cheese prepared with specific cultures that produces particular enzymes that breaks milk proteins to generate above-mentioned peptides will help to control blood pressure. In this context bioactive ‘Val-Pro-Pro’ peptide fraction derived from β-casein and κ-casein are worth mentioning because fermented milks having established antihypertensive attribute containing this peptide are already available in European market.

5. Dairy foods for lowering microalbuminuria

Microalbuminuria (MA) is defined as a persistent elevation of albumin content in the urine of ≥20 μg/min (≥30 mg/d). It is an established risk marker for the presence of cardiovascular disease and predicts progression of nephropathy (kidney disorder) when urine albumin content increases >300 mg/d. Data support the concept that the presence of MA is the kidney's warning to an increased cardiovascular risk. There is a positive correlation between the protein content in the diet and MA. It has been reported that a 0.1 g/kg body weight per day reduction in intake of animal protein was related to an 11.1% improvement in MA. Although beneficial effects from the protein restriction were reported, one study raised concern that too low a protein intake may cause malnutrition. Patients in the low-protein group reported lower energy intakes and a significant decrease in body weight compared to the control group. Therefore, although the majority of the studies report that a reduction of protein to 0.8 g/kg body wt per day may improve MA, this must be done in the context of overall adequate energy and nutrient intake. Increasing the moisture content in products like cheese and paneer and using ultra and microfiltration for selective removal of casein as well as whey proteins could be used for developing lowering protein in dairy products.

6. Lactose intolerance

Lactose intolerance yet another metabolic disorder (but not life threatening) is the inability to metabolize lactose, because of a lack of the required enzyme β-galactosidase (lactase) in the digestive system. Lactose, the major component of milk, is a disaccharide with component monoglycerides, viz., glucose and galactose joined together in β, 1-4 linkage. Lactose as such is rarely absorbed by humans; it relies on the prior conversion to its component monoglycerides carried out by the enzyme β-galactosidase of the mucosal epithelial cells. Because of intestinal β-galactosidase inefficiency, some individuals and even the whole population of some countries, show lactose intolerance and they have difficulty in consuming milk and dairy products. The prevalence of lactose-
malabsorbance is high in East and South India as 60-100% of among the inhabitants are lactose-malabsorbers.

Table 2: Examples of some commercial dairy products with functional or health claim(s)

<table>
<thead>
<tr>
<th>Brand</th>
<th>Type</th>
<th>Source of functionality</th>
<th>Health/function claims</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrifit</td>
<td>Probiotic Drink</td>
<td>Probiotic organisms</td>
<td>Boosting immune system</td>
</tr>
<tr>
<td>b-Activ</td>
<td>Probiotic Dahi</td>
<td>Probiotic organisms</td>
<td>Better digestion &amp; absorption of nutrients</td>
</tr>
<tr>
<td>Amul Prolife</td>
<td>Probiotic Dahi</td>
<td>Probiotic organisms</td>
<td>Healthy digestive system, stronger bones and teeth</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Enhances immune function.</td>
</tr>
<tr>
<td>Amul Calci+</td>
<td>UHT Milk</td>
<td>Added Calcium and Vitamin D</td>
<td>Stronger bones and teeth</td>
</tr>
<tr>
<td>Amul PRO</td>
<td>Malt Based Food</td>
<td>Whet proteins, DHA &amp; essential nutrients</td>
<td>Muscle building, brain development, complete wellness</td>
</tr>
<tr>
<td>Acti-plus</td>
<td>Low fat probiotic dahi</td>
<td>Probiotic organisms</td>
<td>Healthy digestive system</td>
</tr>
<tr>
<td>Yakult</td>
<td>Probiotic Health Drink</td>
<td>Probiotic organisms</td>
<td>Improve digestion &amp; build immunity</td>
</tr>
<tr>
<td>Acti Mind</td>
<td>Milk based Health Drink</td>
<td>Active nutrients</td>
<td>Helps children remain alert &amp; active</td>
</tr>
<tr>
<td>Calpis</td>
<td>Sour milk</td>
<td>Val-Pro-Pro from β-casein and κ-casein</td>
<td>Reduction of blood pressure</td>
</tr>
<tr>
<td>Evolus</td>
<td>Calcium enriched fermented milk</td>
<td>Val-Pro-Pro from β-casein κ-casein</td>
<td>Reduction of blood pressure</td>
</tr>
<tr>
<td>BioPURE-GMP</td>
<td>Whey protein isolate</td>
<td>κ-casein f</td>
<td>Prevention of dental caries, blood clotting, protection against viruses and bacteria</td>
</tr>
<tr>
<td>PRODIET</td>
<td>Flavored milk</td>
<td>αs1-casein f</td>
<td>Reduction of stress effects</td>
</tr>
<tr>
<td>Capolac</td>
<td>Ingredient</td>
<td>Ceseinophosphopeptide</td>
<td>Helps mineral absorption</td>
</tr>
<tr>
<td>PeptoPro</td>
<td>Ingredient/hydrolysate</td>
<td>Casein derived peptide</td>
<td>Improve athletic performance and muscle recovery</td>
</tr>
<tr>
<td>Vivinal Alpha</td>
<td>Ingredient/hydrolysate</td>
<td>Whey derived peptide</td>
<td>Aids relaxation and sleep</td>
</tr>
<tr>
<td>Recaldent</td>
<td>Chewing gum</td>
<td>Calcium casein peptone-calcium phosphate</td>
<td>Anticarcinogenic</td>
</tr>
</tbody>
</table>

7. Dairy foods for the lactose intolerant

Lactose free or low lactose (~30% of normal) dairy products have been developed for the lactose intolerants by partial or complete hydrolysis of the lactose present in milk. The two main methods of lactose hydrolysis are: acid hydrolysis and enzymatic hydrolysis using the enzyme β-galactosidase. The first method is characterized by very severe pH and temperature conditions (pH=1-2, t=100 to 150° C), thus rendering the end product unsuitable for use as a food ingredient. Enzymatic hydrolysis of lactose using β-galactosidase seems to be an attractive method. The enzyme β-galactosidase is widely distributed in nature, e.g., in plants, animal organs, bacteria, yeast and fungi etc. Due to increase expense in using soluble β-galactosidase, the concept of using immobilized enzyme serves as a better alternative to reuse the enzyme, resulting in lowered enzyme cost, besides making the process continuous. Several procedures have been developed for immobilization of enzymes. Adsorption of the enzyme onto an insoluble matrix is the most simple and inexpensive, but suffers from enzyme adsorption due to weak binding. Only very slight improvement could be obtained by cross-linking the enzyme by a
reagent. Gel entrapment, yet another method, where reactor is under diffusion control and thus limited to only small molecular weight substrates. Covalent bonding of the enzyme to a solid matrix is the most important method for immobilization, where enzyme is very strongly attached to the support thus processing a very stable enzyme system.

A range of reduced lactose and lactose free milks are available in American and European Market. The three most popular lactose-reduced or lactose-free products on the US market are Lactacid, Dairy Esse and Mootopia. Lactose free brands available in Europe include Hyla, Emmi, Lacto free, etc. The reduced lactose or lactose free milk manufactured by treatment with β-galactosidase possesses a lower freezing point and sweeter than normal milk. Lactose hydrolyzed milks are more susceptible to Maillard browning during UHT treatment because the mono-saccharides formed from lactose react faster than lactose with amino acids, resulting in extensive browning. The β-galactosidase treatment of fluid milk increases the cryoscopic value of milk from 0.054 to 0.650°C making it difficult to assess the adulteration of milk with water by cryoscopic method. The β-galactosidase treatment increases the cost of fluid milk by ~ $0.06-0.08/L. The dairy company Valio, Finland patented chromatographic separation method to remove lactose from milk. The milk is low in lactose but tastes like normal fresh milk. Examples of some commercial dairy products with functional or health claim(s) are presented in Table 2.

8. Suggested Reading
Recent Applications of Scraped Surface Heat Exchanger in Dairy Food Manufacture

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1. Introduction

Indian milk sweets have played a significant role in the economic, social, religious & nutritional well being of our people since immemorial. The operation flood programme, one of the world’s largest and most successful integrated dairy development programme initiated in 1970, has India to emerge as the largest milk producers in the world. It is estimated that milk production in India reached a record level of 109.4 million in the year 2009 accounting for more than 17% of the world’s total production and about 50-55% of milk produced is converted by the traditional sector(halwais) into variety of Indian milk products. The market of Indian milk product is estimated to be more than 65000 crores.

In view of the growing awareness towards the safety aspects of milk based sweets in India, the consumer shall prefer to buy products from organized sector. Despite the widespread popularity and acceptability of traditional milk products in Indian market, the organized sector has so far not been able to tap into this market potential for many reason such as lack of published literature on their technology, inadequacy of appropriate technology for their commercial production, inadequacy of appropriate packaging material, low keeping quality and lack of quality assurance systems.

Keeping in view the importance of indigenous dairy products and the limitation associated with the existing method, the national commission on agriculture long back recommended that the production of indigenous milk products and sweet derived there from should be taken up by organised plants. The commission also suggested that effort should be made to rationalize the technique of production of various indigenous milk products and explore the possibility of large scale production by improving their keeping quality and packages with minimum expenses.

For handling high viscosity products with or without particles, and for the products that tends to foul of heat transfer surface the scraped surface heat exchanger (SSHE) is most suitable. In SSHE, the working fluid in the form of a film over the heat transfer by rotating blades. Each blade scoops a certain amount of fluid from the pool and accelerates it along the heat exchanger surface. At any given instant the fluid picked up by the form of a fillet in front of blade. The blade action is similar to that of a plough causes part of the fluid in the film to mix with that in the fillet. Simultaneously restoring the film thickness by allowing an equal amount of fluid to squeeze past the tip of the blade. This paper presents the recent innovations on equipment development for manufacture of large scale manufacture of products like burfi khoa, basundi and rabari and ghee

2. Innovative Equipment Development

Based on the work done earlier with two stage SSHE, the design of three stage SSHE was conceived and the equipment was fabricated by M/s SSP Ltd, Fabricated. It consists of three identical thin film scraped surface heat exchangers. The rotor of first two exchanger is identical but the rotor of third stage is altogether different from other two. All rotors of heat exchangers are provided with variable speed drive to run the system at different rotor speeds for achieving desired texture of final product. A feeding tank is provided with
screw pump and changing the speed of impeller provides variation in feeding rate which is measured by magnetic flow meter. In order to make automation in the process the system is provided with instrumentation and process controls. The system is provided with magnetic flow meter, pressure gauges, IP converter, transmitters, pneumatic valves, air pressure indicators, digital panel meter and process controls. A sugar dosing mechanism was also provided at the inlet of third stage to add sugar into the product during its last stages.

3. Method of manufacture of caramelized sugar

Caramelized sugar syrup solution having 50-52% TS was prepared from white crystalline sugar. First of all, White Crystalline sugar 5% w/w of the initial amount of milk was taken in a vessel. It was then heated over the heater with continuous stirring. With passage of time the temperature increased and the sugar started to change its colour. Finally the sugar liquefied and the colour of the sugar turned caramel around a temperature of 160°C. At that point water was added in the ratio 1:1; followed by vigorous stirring to make it a caramelized sugar syrup solution(50-52%TS).

The flow chart described below shows the various unit operation involved:

```
Fresh buffalo Milk
↓
Filtration
↓
Standardization
(Fat/SNF=0.6; Fat=5.1%; SNF=8.5%)
↓
Preheating of Milk ← Sugar (5% of the milk basis), either white crystalline sugar or caramelized sugar syrup solution
↓
Balance tank
↓
Concentration in TFSSHE
↓
Final concentration
(2.5X original TS including sugar)
↓
Cooling (10°C)
↓
Addition of dry fruits and nuts
↓
Basundi (Refrigerated Storage 7 ±2°C)
```

4. Continuous manufacture of Basundi

Basundi was made in three stage SSHE. First the buffalo milk is taken, filtered and standardized to a fat:SNF ratio. It is then preheated to 80°C for few seconds. This Preheated milk is mixed with either white crystalline sugar or caramelized sugar syrup solution in the balance tank. Then the steam valves of the steam header and three stage SSHE, which were located at the rate side of three stage SSHE, which were located at the
rear side of the three stage SSHE, are opened manually. The feed pump is then started and flow is varied between 100-200kg/hr with the help of electromagnetic flow meter by controlling the rpm of feed pump from the control panel. The rotor blade assembly of first and second SSHE is switched on and the speed of both the SSHE’s are kept between 100 to 175 rpm. Milk is first concentrated in the first stage TFSSHE and then enters in to the second stage and where it is further controlled. The mass flow rate is approximately so adjusted to get the concentration required in the Basundi. From second stage SSHE, the product formed is collected and cooled to $10^0$C and then dry fruits were added.

Basundi can be successfully processed using three stage thin film scraped surface heat exchanger. The conclusion of the results obtained from different sets of experiments during studies as follows:

1. Proximate composition analysis, physic-chemical analysis and sensory evaluation analysis reports indicated that Basundi made using three stage TFSSHE was comparable to the mad by conventional method.
2. The scraper blade speed has significant effect on flow rate as well as on the quality of Basundi at constant steam pressure and for constant final product concentration.

The Basundi prepared by using Caramelized sugar syrup solution has the desired caramel colour and flavour, and hence overall consumer acceptability. It can also be analyzed from the table 13 that Basundi prepared by standard batch process had slightly better body and texture as compared to the Basundi prepared in SSHE. The other sensory attributes like flavour and colour and appearance was better in slightly better in case of Basundi prepared in SSHE.

<table>
<thead>
<tr>
<th>Sensory attribute</th>
<th>Standard batch process basundi</th>
<th>Best possible basundi in SSHE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavour(45)</td>
<td>42.01</td>
<td>43.9</td>
</tr>
<tr>
<td>Body &amp; Texture (35)</td>
<td>33.5</td>
<td>33</td>
</tr>
<tr>
<td>Colour &amp; Appearance(15)</td>
<td>13.5</td>
<td>14</td>
</tr>
<tr>
<td>Total score (100)</td>
<td>94.1</td>
<td>95.9</td>
</tr>
</tbody>
</table>

**5. Continuous Rabri making**

First the buffalo milk is taken, filtered and standardized to a 6% fat and 9% SNF. The milk analyzed for acidity to maintain the desired acidity level. If the acidity is lower than desired, the acidity of milk is adjusted by with addition of 2% citric acid solution @0.7 ml per 100 ml milk to raise 0.005unit acidity. Sugar 3%w/w(white crystalline sugar: caramelized sugar syrup solution = 6.27: 1) of initial milk taken is added in the milk in balance tank. Then the steam valves of the steam header and three SSHEs, which were located at the rear side of three stage SSHE, are opened manually. The feed pump is then started and flow is varied between 120-200 kg/hr with the help of electromagnetic flow meter by controlling the rpm of feed pump from the control panel. The rotor blade assembly of first stage and second SSHE is switched on and the scraped speed of first and second SSHE are kept between 100 to 150 rpm (1.968-2.953m/s) whereas third SSHE is kept 15 rpm. Milk is first concentrated in first stage SSHE and then enters into the second stage and finally in third stage sequentially where it is further concentrated and desired flakes formations take place in the product. The mass flow rate is approximately so adjusted to get the concentration required in the Rabri. From third stage SSHE, the product formed is collected and cooled to $10^0$C and packaged in cup then dry fruits were added.
added to it and rabri cup is finally wrapped with aluminium foil. It is stored at
temperature lower than 10°C.

Fresh buffalo Milk
↓
Filtration
↓
Standardization
(Fat=6%; SNF=9%)
↓
The Milk is analysed for acidity to maintain the desired acidity level
↓
Adjustment of acidity with addition of 2% citric acid solution @0.7 ml per 100 ml Milk
to raise 0.005 unit acidity
↓ ← Addition of Sugar (3% w/w on the milk basis) such that white crystalline sugar:
caramelized sugar syrup solution=6.27:1
↓
Mix well Balance tank
↓
Concentration in TFSSHE
↓
Final concentration (56% TS Including sugar)
↓
Cooling (10°C)
↓
Packaging in cups
↓
Garnishing with addition of dry fruits and nuts
↓
Wrapping with aluminium foil
↓
Rabri (Refrigeration Storage < 10°C)

The conclusion of the result obtained from different sets of experiments during studies is
as follows:

1. The Rabri prepared by using 3% level of initially milk taken in combination of
white crystalline sugar and caramelized sugar syrup solution in the ratio of 6.27:1
has the desired caramel colour and flavour and hence overall acceptability.
2. The scraper blade speed has significant effect on flow rate as well as on the
quality of rabari at constant steam pressure and for constant final product
concentration.
3. Body and texture of rabari prepared from three stage SSHe has got better score at
the acidity level of 0.170%LA
4. Acidity has the significant effect on the body and texture of Rabari
5. 1st and 2nd scraper speed have the significant effect on the sensory attributes,
FFA, HMF, colour values (L*,a*,b*), firmness, stickiness of product, Rabari.
6. Consumer acceptability of the product indicates that it was graded excellent by
half of the consumers
7. Mechanization of Rabari can be done using three stage SSHE.
6. Continuous Burfi making

First the buffalo milk was taken, filtered and standardized to a fat 6.0% and SNF 9.0%. This standardized milk to be pre heated to 90°C for few seconds. This milk was mixed with either white crystalline sugar or caramelized sugar syrup solution in the balance tank. Then the steam valves of the steam header which are located at the rear side of three stage SSHE were opened manually. The feed pump was then started and flow was varied between 155-205 kg/hr with the help of electromagnetic flow meter by controlling the rpm of the feed pump from the control panel. The rotor blade assembly of first, second and third TFSSHE was switched on and the speed of all three SSHE were kept fix by control panel. We fix the steam pressure in first, second and third stage 4 kg/cm², 2kg/cm² and 1.5 to 2 kg/cm² respectively. Milk is first concentrated in first stage SSHE and then enters in to the second stage where it is further concentrated. In third stage, we adjust the steam pressure between 1.5 kg/cm to 2.0 kg/cm accordingly by observing the body of the product coming into third stage, from second stage. The mass flow rate is approximately so adjusted to get the concentration required in the Burfi. From third stage, homogenous mixture of final product was collected in well greased plates and spreading into uniform thick layer. Then cooling was done at room temperature and it was covered with aluminium foil. When Burfi got properly cooled, it cut in to pieces and analysis was done.

1. On the basis of preliminary studies it was observed that optimum type and size of grain size was obtained in Burfi by using standardized milk with 0.17% LA initial acidity.
2. In sensory characteristics scores, flavour increases with decrease with scraper RPM, body and texture score is good in medium RPM i.e 150, colour and appearance score is increase with increase in RPM.
3. Grapes and table showed significant treads on the values of hardness, gumminess and chewiness as affected by the different combinations of operating parameters and all others textural characteristics have no significant trends.
4. In three types of sugar/ dosing method, we found very good of overall acceptability in case of addition of crystalline in third stage using scraper RPM 200, 175 and 15 in first, second and third stage respectively. We found best flavour score in case of addition of crystalline sugar and caramelized sugar in 95:5 proportions.
5. Overall heat transfer coefficient increases with increase in scraper speed RPM which varied from 1450 w/m²k to 1775 w/m²k ; 758.36 w/m²k to 1157.71w/m²k and 376.30w/m²k to 451.62w/m²k for first, second and third stage of TFSSHE respectively.
6. Steam consumption increases with increase in scraper speed which varied from 142.45 kg/hr to 116.34 kg/hr; 60.6 kg/hr to 39.69 kg/hr and 11.82 kg/hr to 8.37 kg /hr for first second and third stage respectively.
7. Electric power consumption increases with increase in scraper RPM which varied from 372W to 588W; 360W to 876W; 54W to 192W and 174W to 288W and feed pump motor of TFSSHE respectively.
8. Specific steam consumption was found out to be 1.12 to 0.93 kg steam per kg of milk processing with mean value 1 kg steam per kg of milk processing.
9. Electricity consumption found out to be 6.0 to 8.34 units per 1000 kg of milk process with mean value of 6.7 units per 1000kg of milk process.
7. Continuous Khoa making
The performance of three stage SSHE was evaluated for continuous manufacture of khoa using low fat milk. The standardized buffalo milk having fat percent of 2 to 6% fat was taken and the process parameter and machine parameter of the system were optimised for best product quality in terms of sensory and in terms of physic – chemical attributes. The following conclusions were drawn:

1. Milk having 2, 3, 4 and 6% fat can be successfully processed for khoa manufacture using three stage SSHE.
2. Sensory evaluation and textural profile analysis indicated that khoa made form low fat milk using three stage SSHE is comparable to that made by conventional method.
3. The innovative design of third stage rotor containing two hinged blades in thin film mode and two screw blades with reduced rotor speed (20-30) has improved the textural profile and conveying action of khoa.
4. The process parameter optimisation study indicated that the best quality khoa can be produced by keeping following parameters.
   Mass flow=200kg/h; steam pressure of first , second and third stage SSHE are 4,2, and 1.0 kg/cm²; rotor speed of first, second and third stage SSHE are 200, 200, and 20 rpm.
5. The maximum capacity search trials indicated that milk at the rate of 270 kg/hr can be processed by keeping steam pressure as 5, 3.5 and 1.5 kg/cm² in first , second and third stage SSHE.

8. Continuous ghee making
The potential of three stages SSHE was explored for mechanized manufacture of ghee. The performance of the system was studied to optimize process/machine parameters. The samples collected in this process indicated turbidity in the final product and suspended particle were noticed after clarification. It is suggested that in order to increase size of residue particles, the rotor speed of SSHE be reduced. It was therefore conceived to have new look on rotor design and hinged blade provided earlier was replaced by fixed clearance blade. This investigation was undertaken with the objectives of improvisation of rotor blade assembly for optimizing ghee residue particles during clarification. Ghee made by improvised rotor for which melted butter was used as raw material. The speed of rotor and steam pressure was variable parameter. Trial were conducted at three level of steam pressures 2.5,3.0,3.5 kg/cm² and four level of rotor rpm 70,85,100 and 110. Optimum particle size of ghee residue particles was studied by using centrifugation technique. Quality of ghee was studied in terms of sensory and chemical attributes. The thermal performance of SSHE was determined by collecting condensate. The results obtained from the proposed study have been analyzed through general factorial design and paired t-test, inferences drawn from it. Ghee samples were found completely free from ghee residue particles at rpm 85, steam pressure 3.5 kg/cm² and mass flow rate 300 kg/h and clarification temperature 116°C although capacity of plant was reduced due to decrease in rotor rpm.
9. Continuous butter melter

Butter is basic ingredient for preparation of ghee and melting of butter is one of the important unit operation in continuous manufacture which plays a major role in national economy. Keeping in mind the significance of melting butter, the present study is proposed to improvise the conveying cum mixing rotor in tank type heat exchanger for melting butter so that it can be integrated with continuous ghee making machine for achieving good quality of ghee. The conclusions drawn from the study were as follows:
Conveying commixing rotor in this system for continuous melting of butter was modified with two scraping blades. Molten butter in this process took less time in comparison to conventional kettle process. A capacity around 1010 kg/h has been obtained at 5°C butter temperature with 20 kg butter block.
Overall heat transfer coefficient increases with increase in rotor speed which varied from 210 W/m²K to 280 W/m²K. Higher thermal efficiency of 92% has been found at rotor speed of 270 rpm.

10. Conclusions
The studies carried out have shown that thin film scraped surface has got tremendous potential to manufacture indigenous products in large scale. Three stage thin film SSHE which has been developed by incorporating automation and process controls can produce wide range of Indian milk products by variation in rotor speed and blade configuration. The system can be CIP cleaned and can run six hours continuously with support of power back-up.
Milk Derived Bioactive Peptides-Potential Ingredient for Food Formulations

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Dairy Chemistry Division

1. Introduction

The importance of proteins in the diet has been increasingly acknowledged as a result of new scientific findings in the field of nutrition over the last two decades. The value of proteins as an essential source of amino acids is well documented, but recently it has been recognized that dietary proteins exert many other functionalities in vivo by means of bioactive peptides. These peptides have been defined as specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health (Kitts and Weiler, 2003). Upon oral administration, bioactive peptides, may affect the major body systems, namely, the cardiovascular, digestive, immune and nervous systems, depending on their amino acid sequence. Bioactive peptides usually contain 3–20 amino acid residues per molecule. Such peptides are inactive within the sequence of the parent protein and can be released by digestive enzymes during gastrointestinal transit or by fermentation or ripening during food processing. Milk proteins are considered the most important source of bioactive peptides and an increasing number of bioactive peptides have been identified in milk protein hydrolysates and fermented dairy products. Due to various biological functions milk borne bioactive peptides are regarded as potential active ingredients for preparation of various functional foods, nutraceuticals and pharmaceutical drugs.

2. Bioactive peptides

2.1 ACE inhibitory peptides

They are derived from milk proteins with an affinity to modulate blood pressure have been thoroughly studied in the recent past. Angiotensin I converting enzyme (ACE) is important for blood pressure regulation. ACE also inactivates the vasodilating peptide bradykinin (nonapeptide) and endogenous opioid peptide Met-enkephalin. Many bioactive peptides have been isolated from various dairy products, which have been shown to have an inhibitory, effect on angiotensin I and thereby exhibit antihypertensive activity.

2.2 Opioid peptides

These are derived from food proteins have affinities to bind to opiate receptors and express similar opiate activity, which in turn can be reversed by an opioid antagonist, such as naloxone. Opioid peptides are thought to be biologically very potent; potentially, micromolar amounts may be sufficient to exert physiological effects. Systemic administration of a low dose (1 mg kg⁻¹) of bovine β-casomorphin-5 was shown to improve the disturbance of learning and memory in mice.

2.3 Immunomodulatory milk peptides

They may alleviate allergic reactions in atopic humans and enhance mucosal immunity in the gastrointestinal tract. In this way immunomodulatory peptides may regulate the development of the immune system in new born infants. Milk protein hydrolysates and peptides derived from caseins and major whey proteins can enhance immune cell
functions, measured as lymphocyte proliferation, antibody synthesis and cytokine regulation. The physiological mode of action is not yet known, but they may stimulate the proliferation and maturation of immune system cells.

2.4 Antioxidative peptides

These can be generated from casein by hydrolysis using digestive enzymes or by fermentation of milk with proteolytic Lactic Acid Bacteria (LAB) strains. The identified peptides from yoghurt comprised a few N-terminal fragments of $\alpha_{s1}$-casein, $\alpha_{s2}$-casein, and k-casein, and several fragments from $\beta$-casein. Almost all the peptides identified contained at least one proline residue. Some of the identified peptides included the hydrophobic amino acid residues Val or Leu at the N-terminus and Pro, His or Tyr in the amino acid sequence, which is characteristic of antioxidant peptides.

2.5 Antimicrobial peptide

Antimicrobial activity is usually expressed by the disintegration of cell membrane, whereby the lipid bilayer of the cell membrane is the principle target. Interaction between the antimicrobial peptide and the cell membrane is an important requirement for antimicrobial activity. Milk is a rich source of antimicrobial proteins and peptides, capable of exerting antimicrobial activities comparable to antibiotics. Bovine and human lactoferricin, corresponding to bovine lactoferrin f (17-41) and human lactoferrin f (1-47) display antimicrobial activity against a broad range of Gram-positive and Gram-negative bacteria, including the food pathogen, Listeria monocytogenes.

2.6 Caseinophosphopeptides

Caseinophosphopeptides (CPPs) refer to casein-derived phosphorylated peptides, which contain single and multiple phosphoryl residues and are released by enzymatic hydrolysis of $\alpha_{s1}$-, $\alpha_{s2}$-, $\beta$- and k-caseins both in vivo and in vitro. Several phosphopeptides containing the cluster sequence -Ser (P)-Ser (P)-Ser (P)-Glu-Glu- have been identified from whole bovine casein. These sequences provide the peptides with the unique capacity to keep Ca, P and other mineral in a solution at intestinal pH. The various biological activities of Glycomacropeptide (GMP) - the C-terminal hydrophilic glycopeptide (residue 106-169) released from k-casein by the action of chymosin during cheese making - include its ability to bind cholera toxin and Escherichia coli enterotoxins, inhibition of bacterial and viral adhesion, modulation of immune system responses, promotion of bifido-bacterial growth, suppression of gastric secretions and regulation of blood circulation etc. Owing to its glycoprotein nature GMP has interesting nutritional and physicochemical properties. GMP is rich in branched - chain amino acids (isoleucine and valine) and low in methionin, which makes it a useful ingredient in diets for patients suffering from hepatic diseases. GMP has no phenylalanine in its amino acid composition and this fact makes it suitable for nutrition in case of phenylketonuria. On the other hand, animal model studies have suggested that the high sialic acid content of GMP may deliver beneficial effects for brain development and improvement of learning ability. Bifidobacteria inhibit growth of pathogenic bacteria in the GI tract and are important for the protection of infants from gastrointestinal diseases.

3. Production of bioactive peptides

Technologies for the production of milk protein derived bioactive peptides. The sequences of the bioactive peptides mentioned above are contained within the intact milk proteins and must be released from the proteins by specific enzymatic hydrolysis to extend their health effects. In principle, there are two approaches for releasing peptides from intact milk proteins.
3.1 Enzymatic hydrolysis of milk proteins and enrichment of peptides

One approach is to subject isolated milk protein preparation to hydrolysis in vitro by one or a combination of enzymes containing a great number of peptides among them the bioactive peptides. The hydrolysates or the hydrolysates enriched in particular peptides may be applied in the manufacture of other food products, to provide them with the desired bioactivity. The technological challenges thus lie in the production of milk protein hydrolysates with a high concentration of peptides with a specific bioactivity and with a functionality that makes them suitable as ingredients in other foods, including dairy foods. Hydrolysis can be performed by conventional batch hydrolysis or by continuous hydrolysis using UF membranes. UF steps using low molecular mass cut-off membranes may be useful to separate small peptides from high molecular mass residues and remaining enzymes. After hydrolysis the peptides in the hydrolysates can be fractionated and enriched using different methods. Membranes consisting of negatively charged materials have been used to desalt whey hydrolysates and to enrich cationic peptides with antibacterial properties from cheese whey. Negatively charged CPPs can be isolated by anion-exchange membranes. These techniques provide new possibilities for enriching peptides with low molecular masses and are easily up-scaled to gram or even kilogram quantities. Three different technological hurdles have to be overcome before a product containing bioactive peptides is ready to consider marketing:

- Isolation of the peptides with desired components bioactivity
- Incorporation of the bioactive peptides into a formulated product,
- Verification of efficacy and safety of final product.

Such a sequence of experimental events is also required for the introduction of new food additives; additional is a thorough proof of the claimed benefit of the bioactive peptides. This applies especially when the bioactive component is a completely new substance never consumed before in significant amounts. Due to the economy at scales in dairy industry, it is worthwhile to consider the commercialization of minor components in milk with (potential) biological activity. Separation, purification and production of an industrial level of these peptides must be thought in terms of integrated and high added value use of milk components that is use of controlled separation technologies preserving researched properties, preventing microbial spoilage and taking in account potential value of co-products generated at each step of the used process.

Once a bioactive peptide can be efficiently produced on pilot scale, questions about the relevant properties for application become prominent. It is obvious that there is a desire to have an application range as broad as possible. The peptide will be envisaged in several end products with often completely different product matrices and manufacturing process. To effectively incorporate the bioactive peptides in a product, the prediction of its properties during processing, storage and finally consumer use is required to prevent multiple failures during application development. The first step in this process is the establishment of the key physical and chemical properties of the purified bioactive peptide. This will bring the essential information on parameters such as solubility, stability, heat and pH sensitivity. Step two is the incorporation in the final formulation in a way that the food product is tasteful and the bioactivity maintained in an effective dose. Step three is the ultimate proof that the expected efficacy and safety of the final product can carry the beneficial claims implicitly or explicitly connected to the product.
3.2 Fermentation with lactic acid bacteria

The benefits of the fermented dairy products in the diet are well accepted and the science of food and nutrition has evolved towards ‘nutrition for optimal health’. The central role of microorganisms in fermentation, especially lactic acid bacteria (LAB) is now widely acknowledged, and it is accepted that these microorganisms can exert beneficial effects through two mechanisms: Direct effects or indirect effects during fermentation where these microbes act as cell factories for the generation of secondary metabolites with health promoting properties. Among the latter the most important components in fermented milk are bioactive peptides released from milk proteins by members of the genera Lactobacillus, Bifidobacterium and other lactic acid bacteria.

Lactic acid bacteria (LAB) have a very long history of use in the manufacturing processes of fermented foods and a great deal of effort was made to investigate and manipulate the role of LAB in these processes. For the most extensively studied LAB, *Lactococcus lactis*, a model for casein proteolysis, transport, peptidolysis, and regulation thereof is now well established. In particular, *Streptococcus thermophilus, L. lactis, Lactobacillus helveticus, and Lactobacillus delbrueckii subsp. bulgaricus* (hereafter *L. bulgaricus*) are widely used dairy starters and are of major economic importance. In the milk fermentation processes, the proteolytic system of LAB plays the key role because it enables these bacteria to grow in milk, thereby ensuring successful fermentation. LAB are fastidious microorganisms that require an exogenous source of amino acids or peptides, which are provided by the proteolysis of casein, the most abundant protein in milk and the main source of amino acids.

So the other approach is to exploit the proteolytic system of lactic acid bacteria to partially digest the caseins and whey proteins during the manufacture of dairy products, like fermented milk and cheeses. The technological challenges, thus, lie in the manufacture of fermented dairy products with a high concentration of particular bioactive peptides or their precursors, which upon digestion in the gastrointestinal tract would give rise to the bioactive peptides. The production of bioactive peptides in dairy products is an appealing approach, since this confers an additional positive health effect to dairy product possessing already a health image and having a long history of safe production. During the fermentation of milk and maturation of cheese, the major milk proteins are degraded into a great number of peptides due to the action of indigenous milk enzymes (mainly plasmin), added coagulants and microbial enzymes (especially from starter and non starter lactic acid bacteria, LAB).

The single most effective way to increase the bioactive peptides in the fermented dairy products is to ferment or conferment with highly proteolytic strains of LAB. The challenges in this approach using LAB in dairy products lie in choosing the right strains or combination of strains with optional proteolytic strains of LAB. The strain should not be too proteolytic to destroy the product and yet to give a high proteolysis and with the right specificity to give higher concentrations of the active peptides relative to other peptides i.e. bitter peptides. Moreover the content of potent peptides seems to rely on a balance between their formations and further breakdown into inactive peptides and amino acids, in turn depending on storage time and conditions.

4. Conclusion

As the perception of nutrition expands to encompass the idea of disease prevention and treatment of specific health conditions, diet has become an important element of the mainstream self-care movement. However, for successful future expansion of this aspect
of the functional food market, there is a need to further investigate the range of bioactivities associated with proteins and peptides in food, development of new separation and enrichment technologies, and better techniques for stabilization of the bioactive proteins and peptides in foods. Molecular studies are also required to assess the mechanisms by which bioactive peptides exert their activities in vivo. Much of the appeal of bioactive peptide-containing food products manufactured through dairy fermentation by LAB is the perception that their natural origin equates to a ‘healthier option’. Taking the presented information into account, the future of bioactive proteins and peptides, in health promoting dairy foods and functional fermented dairy foods looks bright. It is probable that, in the future, development of the commercial methods for the isolation of bioactive proteins and peptides, identification of novel bioactive peptides through microbial fermentation and enzymatic hydrolysis of milk proteins will continue, and this practice would be greatly facilitated by the development of high-throughput screening methods based on specific biomarker activity in vitro that can accurately mirror an in vivo health-promoting activity.

5. Selected Reading


Aparna Gupta, Bimlesh Mann, Rajesh Kumar and Ram Bhagat Sangwan (2010) Identification of antioxidant peptides in cheddar cheese made with adjunct culture Lactobacillus casei ssp casei 300. Milchwissenschaft 65:396-399


1. Introduction

Functional foods can be defined as foods containing significant levels of biologically active components that provide specific health benefits beyond the traditional nutrients they contain. The term 'functional food' was introduced in Japan in mid 1980s for food products fortified with special constituents that possess advantageous physiological effects. These types of foods are known in the Japanese market as **Foods for Specified Health Use (FOSHU)**. Among the functional components, omega-3-polyunsaturated fatty acids, conjugated linoleic acid, probiotics & prebiotics, soluble fiber, plant antioxidants, phytosterols, vitamins and minerals, some proteins, peptides and amino acids, as well as phospholipids are frequently mentioned. Functional foods have been developed in virtually all food categories. These products have been mainly launched in the dairy, confectionery, soft-drinks, bakery and baby-food market. This is an attempt to give you a brief about omega-3 fatty acids, conjugated linoleic acid and phytosterols; and their importance as functional food ingredients.

2. Omega-3 fatty acids

Omega-3 fatty acids ($\omega$-3) are a group of polyunsaturated fatty acids (PUFA) which include $\alpha$-Linolenic acid (ALA, C18:3 $\omega$-3), its long chain metabolites Eicosapentaenoic acid (EPA, C20:5 $\omega$-3) and Docosahexaenoic acid (DHA, C22:6 $\omega$-3). The human body is able to synthesize saturated fatty acids such as palmitic acid (C16:0) and monounsaturated fatty acids. However, it cannot produce fatty acids with a C-C double bond in the position $\omega$-3 or $\omega$-6 of the hydrocarbon chain such as linoleic acid (C18:2 $\omega$-6 LA) or $\alpha$-linolenic acid (C:18:3 $\omega$-3 ALA), which are considered essential fatty acids in human nutrition and must be taken directly from diet.

<table>
<thead>
<tr>
<th>Population sector</th>
<th>Recommendations</th>
<th>Expert Committee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy adults</td>
<td>500 mg EPA + DHA / day</td>
<td>ISSFAL</td>
</tr>
<tr>
<td>Pregnant &amp; lactating women</td>
<td>200 mg DHA / day</td>
<td>Perinatal Lipid working group</td>
</tr>
<tr>
<td>Patients with CHD</td>
<td>1 g EPA + DHA / day</td>
<td>American Association Health</td>
</tr>
<tr>
<td>Patients with high triglyceride level</td>
<td>2-4 g/ day</td>
<td>American Association Health</td>
</tr>
</tbody>
</table>

Source: International Society for the Study of Fatty acids and Lipids
2.1 Sources

Major sources of ALA include the seeds and oils of flaxseed, soybean, walnut and canola, while EPA and DHA are obtained in the diet from aquatic and marine products only such as fish, shellfish, algae (*Cryptothecodinium cohnii*) and their oils (Coates *et al*., 2009). Dietary recommendations for omega-3 fatty acids have been given in Table-1.

2.2. Health benefits of omega-3 fatty acids

The health benefits of ω-3 fatty acids (ALA, EPA and DHA) have been widely reported for several conditions including cardiovascular disease, hypertension, atherosclerosis, diabetes, cancer, arthritis, osteoporosis, autoimmune and neurological disorders.

2.3. Incorporation of omega-3 fatty acids in dairy products

The direct use of these omega-3 fatty acid rich oils as a single oil or as blends have certain limitations with respect to sensory qualities, acceptability and reachability. Omega-3 PUFAs are highly susceptible to oxidation due to its highly unsaturated nature. During processing, distribution and handling, omega-3 rich oils can easily oxidize and produce unpleasant tastes and odors, and consequently to a reduction in product shelf life.

A number of companies, including BASF, Roche, Clover, and Ocean Nutrition, manufacture and sell microencapsulated fish oil powders for use in food products. Most of these encapsulated ω-3 oil products are based on the formation of fish oil emulsions using proteins, polysaccharides, lecithin, and other low molecular weight emulsifiers, individually and in various combinations. The emulsions are then spray-dried to form microcapsules. These technologies have allowed the fortification of frequently consumed foods, such as breads, biscuits, soups, yoghurt, cheese, milk beverage, fruit juices, and spreads with reasonable consumer acceptability.

3. Conjugated linoleic acid

Conjugated linoleic acid (CLA) refers to a mixture of positional and geometric isomers of linoleic acid (cis-9, cis-12 octadecadienoic acid) with a conjugated double bond system. The presence of CLA isomers in ruminant fat is related to the biohydrogenation of polyunsaturated fatty acids (PUFAs) in the rumen.

3.1 Sources

The predominant source of CLA in human diets is ruminant-derived food products. CLA is present in milk fat and muscle fat. In the U.S., dairy products provide about 70% of the intake of CLA and beef products account for another 25% (Ritzenthaler *et al*., 2001). The average CLA content of milk fat varies from 0.30 to 0.55 g per 100 g of fatty acids (Dhiman *et al*., 1999). Chin *et al*., (1992) reported that dairy products (milk, butter, cheese and yogurt) and meat contained about 3–8 mg total CLA per g fat. CLA isomers can be prepared commercially by heating linoleic acid (LA) under alkaline conditions or by partial hydrogenation of LA (Banni, 2002).

3.2 Health benefits of CLA

Health benefits of CLA have been attributed to mainly two of its isomers: cis-9, trans-11 (c9t11) and trans-10, cis-12 (t10c12). Rumenic acid has been associated with improving cardiovascular health, anti-carcinogenic properties, improved immune functions and obesity management.

3.3 Incorporation of CLA in milk and dairy products
There are several ways for incorporating CLA into the diet, including manipulating the diet of animals (mostly ruminants) that produce CLA, such as grazing on pasture or feeding with food sources rich in linoleic and linolenic acids as CLA precursors, or by directly enriching dairy products with CLA.

3.4 Enrichment of milk with CLA through animal feeding

Dietary manipulation has been recognized for decades as being the most successful strategy for elevation of the CLA content in milk fat particularly through supplementation of the ruminant diet with fish oils, animal fats, plant oils, synthetic CLA supplements and forage. A number of different plant oils and seeds derived from a range of different sources such as cottonseed, rapeseed, soybean, corn, sunflower, peanut, safflower, canola and linseed have been fed to ruminants in attempts to elevate CLA production. These oils are rich in linoleic acid (cottonseed, soybean, sunflower, safflower, corn) and linolenic acid (linseed), key precursors in the formation of CLA and in particular rumenic acid.

3.5 CLA producing cultures of dairy significance

In recent years strains of a number of dairy starter and probiotic cultures have been identified as possessing the ability to biosynthesize CLA including strains of *Lactococcus*, *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Bifidobacterium*, and *Propionibacterium*. Kim and Liu, (2002) assayed strains of *Lb. acidophilus*, *Lb. bulgaricus*, *Lb. helveticus*, *Lb. johnsonii*, and *Lb. plantarum* for CLA production in both MRS and in whole milk at a linoleic acid concentration of 0.1 mg/ml.

By manipulating CLA content of ruminant milk fat and the identification of CLA producing bacteria (e.g. *Lactobacillus acidophilus*, *Lb. casei*) have opened up avenues for the development of CLA enriched dairy products. Using this knowledge a number of studies have proceeded to produce a range of CLA enriched dairy products, including, UHT milk, butter, yoghurt and in particular cheeses.

3.6 Direct fortification of CLA in milk and dairy products

Conjugated bonds in CLA decrease the oxidative stability of the CLA, resulting in decreased nutritional quality and off-flavor development. CLA is easily oxidized and several studies have suggested that it must be protected until used as a fortifier or additive in foods. Encapsulation is a potential means to transform liquids into stable and free flowing powders which are easy to handle and incorporate into a dry food system. The encapsulation of CLA was reported to increase the oxidative stability. It has been reported that encapsulation can reduce off flavors, allows time releasing of the core and enhances stability of the microcapsules under extreme conditions of temperature and moisture, while being suitable for incorporation into several foodstuffs.

4. Phytosterols

Phytosterols are a group of naturally occurring sterol compounds found in plants that have chemical structures similar to cholesterol, differing only in their side-chain configuration or extra double bond. Their role in plants is similar to that of cholesterol in animals in that they play a vital role in maintaining cellular membrane function. Phytosterols are divided into 2 groups, **sterols** and **stanols**. The 1st group, also called Δ-5 sterols, includes compounds such as campesterol, β-sitosterol and stigmasterol and contains a double bond at the 5 carbon position. In the 2nd group, saturated phytosterols, referred as phytostanols, have no double bond in the ring structure.

4.1 Sources
As dietary sources, fruits and vegetables typically contain only small amounts of phytosterols (less than 0.05% on wet basis). On the other hand, phytostanols occur in certain cereals (wheat, rye, and rice), but their concentrations are much lower than those of unsaturated phytosterols (Moreau et al., 2002). Commercially, phytosterols are obtained following the deodorization of vegetable oils during refining or from tall-oils, which are the fat-soluble hydrolysate fractions obtained during the wood (conifer) pulping process used in paper production (Quilez et al., 2003) and from sunflower oil processing. Overall, tall-oil yields a higher proportion of stanols than vegetable oils, with β-sitostanol being the primary compound.

4.2 Recommended dietary intakes

There have been some differences in opinion regarding the recommended dosages for phytosterols and phytostanols. The United States Food and Drug Administration’s (FDA) suggested that more than twice the amount of stanyl esters (3.4 g/day) than steryl esters (1.3 g/day) were needed to ensure a significant reduction in LDL levels (FDA, 2003). Nowadays, it is assumed that by ingesting 1.5–3 g of phytosterols or stanols, a reduction of 5–15% in total cholesterol and 10–20% in LDL levels can be reached (Normen et al., 2004). Recently, Mensink et al., (2010) found that a daily consumption of plant stanols up to 9 g/day reduces serum LDL concentrations linearly up to 17.4%.

4.3 Health benefits of phytosterols

The use of phytosterols to lower serum cholesterol levels is not a new idea. β-sitosterol, for instance, was used in the 1950s (Pollak, 1953) as a supplement and as a drug to lower serum cholesterol levels in hypercholesterolemic individuals.

4.4 Fortification of phytosterols in dairy products

Since phytosterols are not present in sufficient amount in typical dairy diets, they have to be administered as a supplement. Purified phytosterols that are used commercially are in a highly stable, crystallized form that can take days or weeks to dissolve in bile salt solutions. The solubility of free phytosterols in triglycerides is also low (1% to 2%); however, esterification with long chain fatty acids increases their solubility to levels as high as 10% to 20%. In addition to esterification, products containing free phytosterols can be dispersed in protein, lecithin, or diacylglycerol matrices (Moreau, 2004).

Cargill Company (Cargill Incorporated, USA) intends to market the following vegetable oil derived and tall oil derived phytosterol formulations under the trade name “CoroWise™”. Cargill's phytosterols are primarily composed of β-sitosterol (45%), campesterol (25%), and stigmasterol (25%).

The safety of phytosterol/ phytostanols has been confirmed by government agencies, such as the FDA and in EC by several Commission Decisions (2000/500, 2004/845, 2004/333, 2004/334, 2004/336). In USA (1999), a panel of independent experts concluded that phytosterols were Generally Recognized As Safe (GRAS) for use as an ingredient in vegetable oil-based spreads in amounts that do not exceed 20% (Moreau et al., 2002).

Esterified stanols (derived from both soy and tall oil sterols) has been added into milk, butter milk, yoghurt, drinking yoghurt and cheese spread by various dairy companies. These products are sold under Benecol, Unilever and other brand names. Unilever produces a semi skimmed milk drink containing plant sterols derived from soy and other vegetable oils as well as yoghurt and a mini drink (2 g plant sterols in 100 ml).
5. Suggested Reading


Milk Protein Products for Better Texture of Food Products

Bimlesh Mann, Rajesh Bajaj and Rajan Sharma
Dairy Chemistry Division

1. Introduction
The global demand for milk protein has increased significantly in recent years due to better understanding of its nutritional value, physiological and bioactive properties, and newly developed functional uses. In particular, consumer awareness of the benefits of protein in sports performance, weight management, lean muscle mass retention, satiety and general wellbeing has improved considerably over the last decade. Consequently, the demand for milk protein-enriched food and beverage products has moved into the mainstream as they appear to deliver these benefits more readily than other food proteins. The dairy industry produces a vast range of milk protein products, specifically designed for particular applications. These products include the traditional milk protein products, such as skim milk powder and whey powders, and higher protein products, such as caseins and caseinates, whey protein concentrates and isolates and milk protein concentrates and isolates. Among the higher protein products, milk protein concentrates (MPCs) and whey protein isolates (WPIs) have become very important for the dairy industry, as they provide several processing and functional/nutritional advantages over traditional milk powders.

2. Whey protein
There are three main forms of whey protein that result from various processing techniques used to separate whey protein. They are whey powder, whey concentrate, and whey isolate. The composition of different whey protein forms is given in Table 1. Whey protein powder has many applications throughout the food industry. As an additive it is seen in food products for beef, dairy, bakery, confectionery, and snack products. Whey powder itself has several different varieties including sweet whey, acid whey (seen in salad dressings), demineralized (seen primarily as a food additive including infant formulas), and reduced forms. The demineralized and reduced forms are used in products other than sports supplements. The processing of whey concentrate removes the water, lactose, ash, and some minerals. In addition, compared to whey isolates whey concentrate typically contains more biologically active components and proteins that make them a very attractive supplement for the athlete. Whey Protein Isolates are the purest protein source available. Whey protein isolates contain protein concentrations of 90% or higher. During the processing of whey protein isolate there is a significant removal of fat and lactose. As a result, individuals who are lactose intolerant can often safely take these products (Geiser, 2003). Although the concentration of protein in this form of whey protein is the highest, it often contains proteins that have become denatured due to the manufacturing process. The denaturation of proteins involves breaking down their structure and losing peptide bonds and reducing the effectiveness of the protein. Whey protein contributes low level of fat and lactose to their products which makes them ideal ingredients for formulating sugar free, low fat or non fat dairy products. From functional perspective, WPC and WPI are highly soluble over a wide pH range and contribute emulsifying, water binding, thickening, foaming, gelling, and film forming properties to food and beverages system.
Table 1. Composition (%) of whey protein forms

<table>
<thead>
<tr>
<th>Component</th>
<th>Protein</th>
<th>Lactose</th>
<th>Milk Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey Powder</td>
<td>11 – 14.5</td>
<td>63 – 75</td>
<td>1 – 1.5</td>
</tr>
<tr>
<td>Whey concentrates</td>
<td>25 – 89</td>
<td>10 – 55</td>
<td>2 – 10</td>
</tr>
<tr>
<td>Whey isolates</td>
<td>90 +</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Adapted from Geiser, 2003.

Processed cheese solids were partially replaced by WPC (38% protein) solids by different levels viz. 1.5, 3, 4.5% incorporation of WPC significantly improve body and texture score at 3 and 4.5% level. Processed cheese spread with good melt ability, desired characteristics with improved spread ability can be prepared by using dried WPC at 4.5% of cheese solids (Pinto et al., 2007). By replacing cheddar cheese solids with WPC, firmness decreases significantly while melting quality increases with increase in moisture (Patel et al., 1993). In cheddar cheese, due to incorporation of whey solids the setting time was shorter, and acid development was faster without any significant difference in body or texture characteristics (Rodeney, 1982). In low fat processed cheese, on adding denatured whey protein and co-precipitate at the rate of 10, 20 and 30% give good organoleptic score, good texture, toughness and elasticity (Salem et al., 1987).

Yogurts stabilized with WPC showed more compact structure with more firmness and lower water drainage than control nonfat yogurt. Use of WPC in the manufacture of nonfat yogurt can improve physical and microstructural properties of nonfat yogurt. It can provide a nonfat yogurt with good physical properties that have resemblance to that of full fat yogurt (Aziznia et al., 2008). Yoghurt prepared with MPC and SMP, exhibits higher value of viscosity and more syneresis than yoghurt prepared with WPC (Guzman, 1999). In yoghurt, better sensory, flow properties and greater syneresis was obtained for products prepared with whey powder as compared to those prepared using skim milk powder (Gonzales-Martínez et al., 2002). In yogurts, using 80:20 blends of skim milk solids and sweet WPC have higher gel strength, viscosity and lesser whey separation (Augustin et al., 2003).

Utilizing whey protein in ice cream revealed that the level of total protein, ash, specific gravity, freezing points, viscosity and weight per gallon increased (Shenana et al., 2007). In ice cream, MPC formulation gives higher mix viscosity, larger amount of fat destabilization, homogeneous ice crystal and greater shape retention compared to control (Alvarez, 2005). In ice cream, 1% whey protein hydrolysate gives higher overrun, better melting resistance, sensory and antioxidant properties. The antioxidant activity and overrun is raised by 55 and 26.6% respectively than that of control (Nidhi, 2010). In Khoa, with increased addition of WPC in the milk resulted in large granulation and increased yield (Patel et al., 1993).

3. Milk protein concentrates and isolates

MPC and MPIs range in protein content from approximately 42% to over 90%. Over 60% of the low-protein MPC (MPC 40–59) are used worldwide in dairy products (other than processed cheese). Yogurt and dairy desserts are an application where MPC’s functional properties—improved viscosity, mouthfeel, emulsification, water binding—a favorable nutritional profile and the marketing appeal of a “clean label” increases their appeal. Almost all MPC purchases for use in specialty nutrition (sports, medical and clinical nutrition, geriatric nutrition) are high-protein concentration (80 percent or greater) products. Milk protein concentrate (MPC) powder is basically skim milk powdern with reduced lactose (higher protein) content. Ultrafiltration (UF) is used to remove some
lactose before drying, and UF processing may be adjusted to give MPCs that span a wide range of protein concentrations, for example, 40–85% protein in dry matter. High protein (>70%) MPCs can be slow to dissolve during rehydration and poor rehydration properties negatively affect the use of MPC in many applications. Another serious concern is that high protein MPCs can also exhibit a decrease in solubility during storage especially at storage temperatures > 20°C. MPCs are now commonly added to milk or cheese formulations to enhance the protein content and to improve texture of yogurts. The use of MPCs in nutritional products is growing. In this application, MPC provides both casein and whey proteins in the same ratio as in milk, but without the high lactose content. However, several functionality problems are associated with high protein MPCs, including cold water solubility. Various patented processes have been developed for manufacturing MPC powders with improved functional characteristics, involving alterations in mineral balances and/or the state of aggregation of whey proteins. Development of high protein foods poses several functionality challenges, such as self-association/aggregation and high viscosity of proteins. New types of protein products and associated processes are being developed to overcome some of these technical challenges.

4. Caseinates

Casein is the principal protein found in cow’s milk from which it has been extracted commercially for most of the 20th century. It is responsible for the white, opaque appearance of milk in which it is combined with calcium and phosphorus as clusters of casein molecules, called micelles. The major uses of casein until the 1960s were in technical, non-food applications such as adhesives for wood, in paper coating, leather finishing and in synthetic fibres, as well as plastics for buttons, buckles etc. During the past 30 years, however, the principal use of casein products has been as an ingredient in foods to enhance their physical (so-called functional) properties, such as whipping and foaming, water binding and thickening, emulsification and texture, and to improve their nutrition. Casein is precipitated from the skim milk that is produced after centrifugal separation of whole milk. The skim milk may be acidified to produce acid casein or treated with an enzyme, resulting in the so-called rennet casein. The precipitated casein curd is separated from the whey, washed and dried. Water-soluble derivatives of acid caseins, produced by reaction with alkalis, are called caseinates. Casein is generally not consumed as a food on its own. Casein products are used mainly as ingredients in foods for the purpose of either modifying the physical properties of that food product or providing nutritional supplementation to it. As a consequence, they usually form a relatively minor proportion of the food.

5. Milk protein ingredients for nutritional bars

Texture is a key issue for bars. Texture is multi-dimensional, so formulators need to take into account the initial bar texture and how the texture changes over time, including firmness/hardness, chewiness and degree of tooth-packing. In high-protein bars, the protein source, whether dairy or non-dairy, has a significant impact on bar texture and needs to be chosen carefully to make a product with wide consumer appeal.

Traditional milk protein ingredients for bars include calcium caseinate, milk protein concentrate, milk protein isolate and milk protein hydrolysate. Whey protein ingredients include whey protein concentrate, isolate and hydrolysate. Each ingredient has pros and cons, with many of the cons being flavor-, cost- or texture-related. For example, whey protein hydrolysate forms a soft bar; however, usage levels are limited by its unpleasant flavor and cost. Whey protein isolate has a clean flavor, but its use is limited by chewiness and firmness developing over time. Whey crisps allow formulators to
introduce crunch to bars; this adds interest and can help reduce hardening over time. Whey crisps also contribute a cleaner flavor, when compared to soy crisps. Dairy protein suppliers are creating innovative new protein solutions to reduce limitations. Functionalized milk proteins and whey protein concentrates are now available, and they deliver the same benefits as traditional alternatives, without the limitations, said Marshall. They offer clean, neutral flavors, improved texture and mouthfeel, improved shelf-stability and cost advantages, making it easier to create more appealing bars. For example, a new whey protein concentrate offers a “step change” in bar formulating, enabling more whey to be included in a bar without the extreme chewiness typical of high-whey formulations.

6. Selected Reading


1. **Introduction**

Functional foods have emerged as a new approach to improve human nutrition and well-being. This development has been facilitated by increasing scientific knowledge about the effects of diet and specific dietary components on human health. Accordingly, opportunities have arisen to formulate food products which deliver specific health benefits, in addition to their basic nutritional value. Milk is the source of a wide range of proteins that deliver nutrition to the most promising new food products today. Separation technologies provide the basis for adding value to milk through the production of bioactive components that provide the food industry with nutraceuticals to develop functional foods. The global functional food and nutraceutical market is currently worth about US$50 billion and is growing at some 8 – 10 % annually. This huge and rapidly growing market, driven by consumer demands for health-promoting foods, is creating an almost insatiable desire on the part of food manufacturers for new and novel ingredients with which to formulate these foods. Dairy constituents, notably the milk proteins, provide the food technologist with a rich selection of potential ingredients for functional foods.

2. **Functionality of proteins**

Proteins in food products have both nutritive and technical functionality. Proteins often are added to foods to improve the nutritional quality and, in fewer cases, to exert a physiological activity. Proteins are being used as ingredients in man-made food products because they contribute to one or more of the desired characteristics of that food product. These characteristics might be consumer related (e.g. texture, mouth feel, appearance, taste) or technology related. The latter includes both storage (shelf life, palatability) and processing (e.g. mixing behavior, foam and emulsion or gel formation). Proteins contribute to one or more of these characteristics because of their functional properties. This term is mostly used to indicate physicochemical properties that govern the performance and behavior of a protein in food systems during preparation, processing, storage and consumption. In addition, focusing on proteins as just biopolymers used to create food structures ignores the biological functions of proteins in the diet. These can be beneficial, as in providing amino acids for protein synthesis or bioactive peptides, or these can be detrimental, as in causing a food allergic response.

3. **Why protein rich foods?**

Recent literature suggests that food products with increased protein content have important health benefits. Several nutritional studies showed that proteins have stronger satiating effect as compared to carbohydrates and fats. Intake of protein-rich foods give a stronger feeling of satiety compared to a carbohydrate- based product with the same caloric value. Intake of dairy fruit drink enriched with protein before lunch, triggers less energy consumption at lunch compared to the same drink enriched with carbohydrate. Whey protein seems to induce a satiety signal that influences both short term and long-term food intake. Lower subsequent food intake due to high-protein diets led to a statistically significant decrease in total body weight in case of long term diets (6 months...
or longer). Also, high protein diets result in increased energy expenditure following the consumption than low protein diets. Therefore high protein foods are considered to be a potential candidate for body-weight control and treatment of obesity. According to the RDA recommendations, our protein requirement is of 0.8 to 1.0 gram of protein for every kilogram of our body weight daily. Roughly, the protein requirement is about 10-15% of calories coming from protein daily. The protein requirements for sport athletes like runners, cyclists and triathletes are from 25 to 50% more (0.5 to 0.72 gram of protein per pound of body weight) than for non-active individuals. The recommended dietary allowance of proteins, i.e., 0.8 g/kg body weight is generally not enough for elderly people to maintain their fat-free mass, in particular their muscle mass. Protein intake larger than the RDA, may help against chronic wasting (loss of muscle mass), an important aging-related disease. It is also reported that higher protein intake may have positive effects on the bone health of the elderly.

4. Challenges in development of protein rich functional foods

Protein-rich foods generally have low stability. The stability problem arises because protein-protein interactions upon storage may give rise to strong and tight networks, product hardening and syneresis of water. The additional formation of intermolecular disulphide bonds and/or non-covalent interaction lead to increased protein aggregation. The changes in protein-rich product properties seem unavoidable, because they are related to inherent properties of proteins. The product hardening is further favored by moisture-redistribution and the Maillard reaction. This reaction, initiated by the reaction between reducing sugars and amine groups in proteins, contributes to the formation of covalent bonds between protein molecules resulting in hardening during long-term storage. The physicochemical changes in properties of protein-rich products thus lead to changes in texture and, subsequently, the sensory properties. These physical-chemical changes also depend on the protein sources added to the product. As an illustration, the addition of whey protein in meal replacement bars (100% whey bar) gave dense products with strong cohesiveness and adhesiveness. The use of soy protein (100% soy bar), however, led to products that were less dense, more sticky and showed increased hardening in time. Thus, the use of a high concentration of globular protein in the native form to create high-protein foods is challenging, because above the critical concentration of whey protein, i.e., 12% w/v, gelation may already occur below the denaturation temperature.

5. Approaches in production of protein-rich foods

Protein-rich foods are often produced using a thermal treatment, leading to protein denaturation. Highly concentrated proteins dispersion might give rise to a dense network, which becomes even denser at elevated temperatures. The product texture is often softened using some ingredients. The use of whey protein hydrolysate instead of whey protein isolate in nutritional bars leads to a softer and more flexible product, and lowers the hardening to a minimum over time. The protein matrix in low-fat cheeses has low porosity leading to a more compact matrix. As a result, the cheese becomes more viscous and, hence, less spreadable. It also has an increase in firmness and fracture stress compare to full-fat cheese. A great variety of ingredients has been investigated to improve structures and textures of low-fat cheese by increasing the moisture content, for, e.g., by using fat replacers and exopolysaccharide (EPS)-producing starter culture.

6. Types of processing techniques

Different processes exist for creating protein-rich foods with good microstructure, such as extrusion and spinning. Extrusion of protein is used to texturize the protein (in case of a
protein-based product), to increase protein content in the end product (in case of a protein-enriched product), to create an anisotropic structure, and probably to obtain better expansion in the case of porous products. Most of the studies have been done using a twin-screw extruder for texturization of whey protein. Initial mixing of whey protein with 40% moisture content in the extruder, lead to paste or slurries with irregular aggregates (string and granules), ranging from 10 to 200 nm. The extruder-denatured insoluble protein shows a dense and compact structure at nanometer scale.

7. Novel processing concepts for structuring protein-rich foods

Trends of new design technologies for structuring protein-rich foods involving the creation of heterogeneity by pre-texturization and the use of structure elements may help to solve the problems of protein-rich foods. The hardening of protein-rich bars involves solvent rearrangement, disulphide bond formation, non-covalent interactions, and Maillard reaction. Plasticizers are often used to soften the products and to retard the hardening. Structural element is defined as domains (from only protein or in combination with other ingredients), in the shape of particles, fibers, etc. in the size range of nm until mm. The use of structural elements may play an important role to create desirable structures from concentrated globular proteins. Pre-texturization induces a first change in protein properties with process conditions that differ from the final structuring process. Unfolded state of whey protein can be achieved through shear at 50°C, which is well below the denaturation temperature of 70-80°C. Further, a number of methods exist to form small particles that can be applied to create protein-rich structure elements. These methods are microparticulation, double emulsification, or in combination with other biopolymers through phase separation. These options are mainly explored to create products with a dispersed protein-rich phase.

8. General nutritional role of milk proteins

The nutritive value of milk proteins may be assessed by (i) the energy they supply (ii) their digestibility and absorbability (iii) their content of essential nutrients, i.e., essential amino acids, associated fatty acids, minerals and vitamins, and (iv) their allergenic potential. With allowances for incomplete digestion and the fact that part of the milk protein is converted to and excreted as urea, the metabolically available energy from milk protein was estimated to be 17 kJg⁻¹. Protein quality may be expressed by a number of terms including biological value (BV), protein digestibility (PD), net protein utilization (NPU), protein efficiency ratio (PER) and protein digestibility corrected amino acid score (PDCAAS). Compared to whole milk and casein, whey protein has higher BV, PD, NPU and PER values. The nutritive value of the different milk protein fractions primarily depends on their essential amino acid content. Both human and bovine milk proteins are an excellent source of essential amino acids. Furthermore, due to the fact that caseins are rich in tyrosine and phenylalanine and whey proteins are rich in cysteine and methionine both fractions combined have a greater protein quality than either alone due to their complementary concentrations of amino acids.

9. Role of dairy proteins as nutritional supplements

A substantial difference exists in the amounts of digestible amino acids supplied by plant proteins (e.g. soy) and milk proteins (e.g. α-lactalbumin). The often first-limiting amino acids (lysine and methionine plus cysteine) are found in much higher concentrations in the milk proteins, making them excellent sources of amino acids and very important dietary constituents to afford a balanced dietary protein intake. Because of their relatively high levels of nutritionally important amino acids, milk proteins are utilized efficiently by
humans, when given as a sole protein source. Given the high bioavailability of amino acids in milk proteins and their abundant supply, it is hardly surprising that milk proteins are commonly used for the manufacture of so-called nutritionals, i.e. foods designed for a specific nutritional purpose (e.g. infant, sports, elderly formulas).

10. Role of dairy proteins in specific physiological roles

Amino acids may have physiological roles that are unrelated to their direct involvement in protein synthesis. These include their roles as neurotransmitters (e.g. glutamate, aspartate and glycine) and as precursors for the synthesis of other molecules involved in neuromuscular function (e.g. creatine and taurine) and in host defenses (e.g. glutathione and nitric oxide). Tryptophan is a precursor for the synthesis of serotonin, potentially impacting mood control and appetite regulation, whereas the nitrogen-rich amino acid arginine leads to the production of nitric oxide, which is considered to have a significant role in cell signaling and the control of endothelial tone. Depending on its site of release, nitric oxide exerts several known functions, including stimulation of the pituitary gland, vasodilation, neurotransmission and immune modulation. Another example of an amino acid with a specific metabolic role is the branched chain amino acid leucine, which has a unique role in the regulation of muscle protein synthesis. Interestingly, leucine stimulates protein synthesis directly in skeletal muscle but not in liver. The other branched-chain amino acids—iso leucine and valine—are less effective in stimulating muscle protein synthesis compared with leucine. Leucine supplementation has been shown to stimulate recovery of muscle protein synthesis during food restriction and after endurance exercise. It has also been suggested that leucine has a potential regulatory role in glycaemic control. There are also amino acids not found in proteins (i.e. non-protein amino acids) with specific physiological functions. The classic example is taurine (\(\beta\)-aminoethanesulfonic acid), which is synthesized by the body from cysteine or methionine and is essential for the production of conjugated bile salts (taurocholic acid). Taurine is found in milk, normally in the free form. It is recognized that cow’s milk has low concentrations of taurine relative to human milk.

11. Role of dairy proteins in design of weight loss foods

Dairy proteins are a highly versatile source of amino acids, for the design of weight-loss foods. It is now widely accepted that protein is a satiating nutrient and is effective relative to carbohydrate and fat in suppressing voluntary food intake independent of its calorific value. The role of dietary protein in the regulation of food intake and body weight in humans, and underlying mechanisms, has been the subject of recent reviews. There is strong evidence that the protein content of a food is a determinant of short-term satiety and of how much food is eaten. The role of protein in the regulation of long-term food intake and body weight is less clear, because of a paucity of relevant experimental observations. The role of protein in body weight regulation, in comparison with other macronutrients, is considered to consist of several often-related but different aspects: satiety, thermogenesis, metabolic energy efficiency and body composition. As stated, protein appears to increase satiety and therefore helps to sustain reduced-energy-intake diets. Firstly, the highly satiating effect of protein has been observed both post-prandially and post-absorptively. Secondly, and also as discussed, high-protein diets are associated with a high dietary-induced thermogenesis, which could be related to the satiety effect of proteins. Thirdly, high-protein diets assist to maintain or increase fat-free body mass and the maintenance of a higher lean mass is costly energetically (i.e. a higher resting energy expenditure), leading to a lower associated metabolic efficiency of energy utilization. With special reference to the dairy industry is the observation that protein source per se
may be a factor influencing short-term satiety in humans. Whey protein has been identified as a candidate protein that may be highly effective in promoting satiety. A basis for differences in satiety related to source of protein may be found in amino acid composition (e.g. a high leucine content stimulating protein synthesis and altering body energetics), in bioactive peptides released from the protein during digestion (refer to the following section), in different kinetics of protein digestion and, in the case of whey, in the presence of glycomacropeptide.

12. **Milk proteins as a source of bioactive peptides**

Most of the bioactivities of milk proteins are latent in the original native protein. Among the potentially greater significance, however, are the many small (from 3 to 20 amino acids) bioactive peptides encrypted in food protein amino acid sequences and released during digestion. Bioactive peptides are specific protein fragments that influence body function. These peptides are inactive within the sequence of the parent protein and can be released during proteolysis or fermentation. Bioactive peptides may act as physiological modulators locally in the gut and, potentially, systemically. Most, if not all, proteins appear to contain bioactive sequences, although the majority of research to date has been conducted with milk.

13. **Milk protein as source of immune factors**

Milk is known to contain proteins (e.g. lactoferrin, lactoperoxidase, immunoglobulins) and free peptides having specific non-nutritional physiological functions. These compounds are undoubtedly important in the case of the human infant and may also have a functional role in adults. Colostrum is a rich source of immunoglobulines, IgA and IgG in particular but also IgD, IgE and IgM. Bovine colostrum has been proved not only to support the body’s immune system, but also contains valuable growth factors that promote tissue repair (Cara, 2008). Colostrum powder contains a combination of bioactive components to support a healthy immune system. Lactoferrin (an iron-binding whey protein found in naturally occurring body fluids, i.e. tears, saliva and breast milk) has the highest concentration in colostrums. It is a major constituent of leukocytes and owing to its iron-binding properties is able to bind pathogens invading the body, thus having an antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant and immunomodulatory activities. Colostrum also contains lysozyme and lactoperoxidase. Lysozyme (sometimes referred to as the body’s own antibiotic), which has the ability to destroy cell walls of certain bacteria and lactoperoxidase, also an enzyme, possesses antimicrobial properties. More recently, the application of commercial ultrafiltration (UF) to bovine colostrum has resulted in the creation of powdered food ingredients which contain as much as 80% (w/w) protein and 50% (w/w) IgG. The obvious difference in Ig form notwithstanding, bovine colostrum-derived IgG concentrates intended for human consumption have an annual market of at least $100 M USD, with the material used as a dietary supplement ingredient in sachets, capsules, tablets, and liquid supplements and as an infant formula ingredient. This market for IgG concentrates has transformed bovine colostrum from an agricultural by-product into a valuable raw material.

14. **Conclusion**

Concentrated protein-rich foods have strong potential to be developed in terms of health and well-being roles. The exploitation of high nutritional value of milk proteins offers a number of opportunities in the development of protein enriched functional foods including specific nutritional, pharmaceutical and medical foods. Technological
interventions in creating products with the right texture and stability will overcome the hindrance in the use of these products by consumers.

15. **Selected Reading**


Recent Trends in Bio-functional Dairy Beverages

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1. Introduction
During the last decade, consumers approach to healthy foods has changed dramatically, and today enhancing the health span of consumers through consumption of healthy food is more important than simply enhancing their life span. Rising medical costs are the prime factor forcing people to find cheaper and effective means of protecting their health. This fact has led to an increase in consumer’s interest in functional foods. Dairy products occupy a significant space in the functional foods market and dairy-based functional beverages are a growing segment of this sector. Functional foods and beverages are the foods containing health promoting components that extend beyond traditional nutrients. Convenient and good-tasting products having functional properties are now very successful. Consumers are also looking for ways to include more healthy food products in their diets. A functional beverage is only successful if the product tastes good, is convenient to consume, has a clear message and can be bought at an affordable price. Functional beverages having both heath benefits and good flavour have become increasingly popular in recent years. Functional drinks appeals the consumer by their special properties like boosting the immunity system, relieving stress, increasing vitality and stamina, improving intestinal health, controlling cholesterol and body weight and fighting degenerative diseases. Functional beverages are drinks that have been enhanced with added ingredients to provide specific health benefits beyond general nutrition. Functional beverages includes sports drinks, energy drinks, functional drinking yoghurt, bottled water, hot drinks, concentrates, tea, coffee fruits and vegetable juices . Energy drinks and sports drinks are on high demand by the consumer.

2. Functional dairy-based beverages
Functional dairy beverages Supermarket shelves in Europe, America and Japan carry a wide range of functional dairy beverages with probiotics, prebiotics, omega-3, plant sterols and many other components now a days. In the past couple of years a large numbers of functional dairy beverages containing a wide range of non-dairy bioactive additives have been launched in Europe, Japan and America. Tea is one the most commonly used dairy beverage in the world having ingredients which give beneficial health impacts.

3. Probiotic dairy beverages
Consumption of probiotic bacteria via food products is an ideal way to reestablish the balance of intestinal microbiota. These include alleviation of lactose intolerance symptoms, lowering cholesterol, curing antibiotic-associated diarrhea, prevention of intestinal tract infections, prevention of colon cancer, control of rotavirus, prevention of ulcers related to Helicobacter pylori, improvement of immune system, irritable bowel syndrome and antihypertensive effects. In order to produce therapeutic benefits, a suggested range for the minimum level for probiotic bacteria in probiotic milk is from $10^6$ to $10^7$ colony-forming units (cfu)/ml. In recent years, probiotic beverages based on fruit juice, cereal products and daily dose dairy drinks have also become popular.
commercially. Today, a wide range of probiotic products is available for consumers in the market. Dairy products have a distinct role in delivering probiotic bacteria to the human gut, as these products provide probiotic bacteria with a suitable environment in which their growth and viability are promoted. In recent years, probiotic beverages based on fruit juice, cereal products and daily dose dairy drinks have also become popular commercially. Among the probiotic bacteria used in the manufacture of dairy beverages, Lactobacillus rhamnosus GG is the most widely used. Lactobacillus rhamnosus GG is acid and bile-stable, has a great avidity for human intestinal mucosal cells, and produces lactic acid. The Gefilus product range includes yogurt, milk, buttermilk and drinks. AKTfit, Biola, BioAktiv, YOMO, LGG+, Yoplait360, Kaiku Actif are among the other commercial dairy-based functional beverages containing Lb. rhamnosus GG. A dairy-based probiotic beverage fermented by a mixed culture of Lactobacillus casei Shirota and Bifidobacterium adolescentis containing 35% buffalo cheese whey, 30% soy milk and 35% cow’s milk has been developed.

4. Non-probiotic dairy beverages with added bioactive components

Bioactive substances of food origin are considered to be dietary components which exert a regulatory activity in human organisms beyond basic nutrition. It is a well established fact that food proteins, especially milk caseins, may act as precursors of biologically active peptides with different physiological effects. Nutraceuticals, i.e. omega-3 fatty acids, isoflavones, and phytosterols have positive effects on human health. Among nutraceuticals, Omega-3 fatty acids, a group of healthy lipids, as a functional ingredient has a wide range of health benefits, including reduced risk of cardiovascular diseases and certain cancers. Omega-3 is considered an essential nutrient since it is the precursor of EPA and DHA, which cannot be synthesised in the human body and are essential for development of the brain, concentration, and the learning ability of children, as well as promoting health in the general population. The addition of Omega-3 fatty acids to probiotic yogurt causes no aroma and flavour defects in set type products. Some reported functional properties of dairy beverages are as follow:

- Promote the reduction of blood cholesterol
- Prevent certain illnesses, such as cardiovascular disease
- Visual development in children
- Improve immune reactions against allergies
- Reduce the risk of forming blood clots
- Boost the immunity system
- Reduce the effects of lactose intolerance and constipation

5. Functional whey-based beverages

Whey is a byproduct of the process of cheese production. Liquid whey consists of approximately 93% water and contains almost 50% of the total solids present in milk, of which lactose is the main constituent. The nutritional and techno-functional properties of cheese whey have demonstrated that whey is a valuable source for highly prized nutraceutical ingredients such as lactoferrin, lactoperoxidase, immunoglobulins, growth factors, etc. These findings have triggered scientific efforts to convert cheese whey from a waste product into one with added value. So far a great number whey-based beverages has been presented to the markets. Whey-
based products can be grouped as follows: Dairy-type beverages, thirst-quenching beverages and fruit juice-type beverages. Keeping in view increased demand of soft drinks and juices these days in India, there is a tremendous scope and need to exploit commercial production of these fermented whey beverages since it is the best proposition to convert largest by-product (whey) of dairy industries into value added product by simple and indigenous processes.

Dairy-based whey drinks rely on the fermentation of liquid whey protein concentrate or the enrichment of milk and milk products with dry whey protein concentrate (WPC) and whey protein isolate (WPI). During the last decade special attention has been paid to the production of fermented whey beverages with probiotic bacteria. A fermented whey drink 'Acido whey' was produced by probiotic \( L. \) acidophilus and patented. A whey beverage was developed using \( Lb. \) rhamnosus NCDO 243, \( Bifidobacterium \) bifidum NCDO 2715 and \( P. \) freudenreichi subsp. shermanii MTCC 1371. The product was produced by adding 4% of mixed culture(1:1:1) into deproteinised whey (4.6% lactose, 0.62% ash, 0.48% fat and 0.5% protein) adjusted to pH 6.4 and incubated at 37°C for 8 h. The counts of probiotic strains were high enough for therapeutic effect in the product after 15 days of storage, with no organoleptic defects. Most recently have developed a fermented probiotic beverage from milk permeate enriched with whey retentate using \( Lb. \) acidophilusM92, \( Lactobacillus \) plantarumL4 and \( Enterococcus \) faecium L3. Hernandez-Mendoza et al. (2007) developed a whey-based probiotic product made by inoculating reconstituted whey containing pectin and sucrose with \( Lactobacillus \) reuteri and \( Bifidobacterium \) bifidum. After 30 days of storage, the product retained an acceptable flavour as well as probiotic bacteria counts higher than 106cfu/mL. Similarly, the survival rate of commercial probiotic bacteria (\( Lb. \) Acidophilus LA-5 and \( Bifidobacterium \) animalis subsp. lactis BB-12) in reconstituted whey beverage throughout 28 days of storage was satisfactory. A fermented probiotic beverage was produced using Minas frescal cheese whey by Almeida et al. (2008). The acidification rates of \( Lactobacillus \) delbrueckii subsp.bulgaricus, \( Lb. \) acidophilus, \( Lb. \) rhamnosus, and \( B. \) animalis subsp. lactis in co-culture with \( Streptococcus \) thermophilus showed differences, being slowest in the \( Str. \) thermophilus and \( Lb. \) rhamnosus mixture. The whey beverages proved to have market potential as far as technological and sensory properties were concerned. Recently, a biofunctional strawberry probiotic whey drink was prepared using yoghurt culture and probiotic culture of \( L \) acidophilus which has antimicrobial, antihypertensive and antioxidant properties. Another whey drink was prepared using \( L \) acidophilus, a probiotic culture which was able to control diarrhoea in mice model.

6. Benefits of whey based fermented drinks

Whey is an excellent growth medium for Lactic Acid Bacteria to ferment lactose in whey to form lactic acid. Whey is a genuine thirst quencher unlike most of the soft drinks. Whey as a drink can replace much of the lost organic and inorganic salts to the extracellular fluid. Whey is rapidly adsorbed due to absence of fat emulsion. This has been used to treat various ailments such as arthritis, liver complaints and dyspepsia. It also possesses almost all the electrolytes of Oral Rehydration Solution (ORS) which is invariably used to control dehydration. On fermentation with LAB, it becomes a suitable drink for lactose-intolerant people. Fermentation of whey with LAB also masks the effect of curdy flavor of whey. At industrial scale, large volumes of whey can be used directly from paneer/cheese vats, thus eliminating transportation and disposal problems. Conversion of whey into beverages involves very simple processes. Utilization of whey
generates additional revenue to the dairy plant. Above all, its utilization also solves the problems of environmental pollution.

7. Other fermented dairy products

7.1 Fruit Lassi

Cultured dairy products are an excellent medium to generate an array of products that fit into the current consumer demand for health-driven foods. Owing to expanding market share and size of dairy companies, there has been a reduction of clearly structured markets i.e. merging of dairy products and fruit beverage markets with introduction of juiceceuticals that include hybrid products like fruit based cultured milk beverages. In India lassi made out of dahi is a widely consumed fermented milk beverage, popular in all parts of the country and has a great potential in the domestic as well as overseas markets. However, problems like short shelf life, post acidification, whey syneresis, hinder the market saleability of lassi. Accordingly, studies were conducted for the production of fruit lassi with extended shelf life using biopreservatives. Khurana (2006) developed a process for making fruit lassi, using buffalo milk. Standardized buffalo milk was heated to 90°C for 10 minutes followed by cooling to 30°C and inoculated with 1.5 per cent culture and incubated at 300C for 10-12 hrs. The curd obtained was then mixed with sugar syrup and fruit pulp. Homogenization was done followed by packaging, cooling and storage at 4°C. For further stabilization and improvement of consistency of each type of fruit lassi exopolysaccharide producing (EPS+) cultures were used along with pectin at different levels. An enhancement in the rheological and overall sensory characteristics of all the 3 types (banana, mango and pineapple) of fruit lassi was observed with increase in proportion of EPS+ culture up to a certain level. Nisin and MicroGARDTM were used to extend the shelf life of fruit lassi up to 30 and 50 days, respectively as compared to control mango lassi which had a shelf life of 15 days at 4±1°C. The technology developed for manufacture of fruit lassi with extended shelf life appeared to have considerable potential to facilitate commercial manufacture and marketing of this popular fermented milk beverage. Inclusion of fruits and artificial sweeteners in lassi would not only enhance nutrition, help diabetic/obese people, aid product diversification but also help in curtailing the post harvest losses in fruits. Such a product would not only serve as a low calorie-quick meal snack but also offer stiff competition to expensive soft drinks in the beverage market.

8. Cereal based fermented dairy beverages

Cereal grains constitute a major source of dietary nutrients all over the world. Addition of cereals into milk not only enriches its mineral value but also supplements fiber. Fermentation further enhances the nutritive value, palatability and functionality of cereals by reducing the antinutritional factors. Development of technologies for the manufacture of cereal based fermented milk beverages will lead to the utilization of underutilized cereals like sorghum, pearl millet, finger millet etc. Raabadi, prepared by cooking cereal (maize) flour with buttermilk, is a traditional popular beverage of Haryana, Punjab and Rajasthan. The technology of producing these traditional fermented foods from cereals and milk remains a household art. Traditional process of raabadi preparation yields a product with limited shelf life (one to two days) with unpredictable sensory quality. A technology has been developed at National Dairy Research Institute, Karnal for manufacture of raabadi-like fermented beverage using cereals like Pearl millet/Sorghum/wheat possessing health attributes. Development of raabadi-like fermented milk beverages was based on the selection of milk solids source; selection of levels of milk solids and cereal solids, stage of addition of cereal solids addition;
fermentation conditions and stabilization of developed product in terms of preventing sedimentation and wheying-off in the product during storage. Cereal solids were incorporated in milk at two stages i.e. before fermentation with dahi/yoghurt culture or after fermentation. The fermented mass will be blended into beverage by the addition of water, salt and spices. The cereal based beverage thus developed was having very good sensory qualities and it was accepted well by the consumers but the only limitation of the beverage was its limited shelf life. Attempts were made to increase the shelf life of the product in which, this beverage was made in ready to reconstitute form Thus, it not only increased the shelf life of the beverage but at the same time it aided to the convenience in use. In other study to increase the shelf life of the liquid beverage, various preservatives viz. biological, chemical and thermal treatments were tried and the shelf life increased to about a month at refrigerated temperature. Gupta et al. (2007) prepared raabadi from buttermilk and mothbean by two methods. Buttermilk was mixed with mothbean flour to get homogeneous paste and this was diluted with plain water and boiled for 7 min with constant stirring while salt was added during stirring. In the first method the mix was fermented before cooking and in the second method the mix was fermented after cooking. They reported that 4 h fermented and cooked and 12 h cooked and fermented raabadi had better acceptability. Kindumu is a fermented milk beverage popular in central African region and is prepared by sun drying the mixture of fermented milk and germinated/non-germinated sorghum flour. Grewal and Chauhan (1993) prepared Soy raabadi by blending autoclaved dehulled soybean slurry and the curd mass (obtained from reconstituted skim milk). The blend was added with water and fermented at 30°C for 12 h. Soy raabadi appeared to be a low cost wholesome food which was free from beany flavour and rich in protein, fat and dietary essential minerals. Mugocha et al. (2000) developed a composite finger millet and milk based fermented beverage. Various parameters including level of finger millet gruel in skim milk, type of starter culture, incubation temperature etc, were optimized to develop the finger millet dairy beverage.

9. Selected Reading

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Mode of Action of Probiotics in Humans

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1. Introduction
Probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit on the host (WHO/FAO 2002). In the decade (last decade) after the acceptance of this universal definition, the scientific community worldwide worked intensively to elucidate the various mechanisms of probiotics mode of action that benefits the human beings in the maintenance of health and well being. Several of these mechanisms are related to improved nutritive status of the target host, immunomodulatory and anti-inflammatory effects against various allergic and inflammatory disorders, antimicrobial effects against potential intestinal pathogens, improved intestinal barrier function in the prevention and treatment of various gastrointestinal diseases, improved energy and glucose metabolism in the management of lifestyle diseases. All these established mechanisms with strong scientific data generated from various in vitro, in vivo animal and human clinical trials attracted various industrial partners to invest in the formulation of probiotic foods to attract and meet the needs of health conscious consumers in the global market. With these combined approach, today probiotic foods share the hottest market worldwide in the functional foods category.

2. Probiotics in improving Nutritional status of host
The role of probiotics in improving the nutritional status among people of different age groups (neonates and adults/elder) and health status (diseased/normal) have been extensively studied by various investigators in the last few decades (Fukushima et al., 2007). The main mechanisms involved in improvement of nutritional status of host are: 1) improved gut microbial balance for effective metabolism of nutrients, their absorption and bio-availability 2) improved synthesis of bioactive components like organic acids, bioactive peptides, short chain fatty acids, vitamins which contribute significantly towards the betterment of nutritional status of the host. Recently, the application of ‘omic sciences’ in nutritional research have unraveled various signaling pathways which are modulated by probiotics and their metabolites that elicit specific responses in the host (Bronet et al., 2012).

3. Probiotics against allergic and inflammatory disorders – possible mode of action
The capacity of probiotic bacteria to modulate immune responses is widely used as strategic approach to treat various allergic and inflammatory disorders (Klaenhammer et al., 2012, Jan et al., 2012). The main mechanisms of probiotic action on immune status and inflammatory processes of host have been well documented backed up with cytokine production profiles in various in vitro cellular and in vivo animal models and their efficacy has been established with intervention studies in human clinical trials. The mechanism of immunomodulation involves the secretion of IgA that provide the host with a first line of immune defense, modulation of DC/NK interaction, maintenance of Th1/Th2 immunity and enhancement of Treg activity to modulate cytokine secretion.

The role of probiotics in the intestinal regulation of inflammatory processes has also been thoroughly investigated and hence these organisms can be explored as biotherapeutics in...
the management of multitude of disorders both inside and outside the gut. The main mechanisms involve the attenuation of LPS induced inflammation through the down regulation TLR4/NF-kB that secretes pro-inflammatory cytokines (Lee et al., 2012)

4. Probiotics and improved intestinal barrier function

The intestinal barrier plays an important role in the maintenance of human health by protecting the host from penetration of dangerous macromolecules like LPS besides nutrient absorption. The intestinal barrier is a complex multilayer system made up of an external "anatomic" barrier and an inner "functional" immunological barrier. The interaction of these 2 barriers enables equilibrated permeability that helps in maintenance of human health. However, the factors like gut microflora modifications, altered mucus secretion and epithelial damage were found responsible for decreased barrier function of intestine and increased translocation of luminal contents to the inner immunological barrier. The increased translocation of luminal contents through leaky epithelium causes overstimulation of inner mucosal immune system that is responsible for development of various pathophysiological consequences resulting into diseases or disorders like IRD, IBS, colitis and Metabolic Syndrome (Scaldaferri et al., 2012).

Probiotics are the attractive therapeutic option for improving the gut barrier function through the modulation of gut microbial composition, increased mucus secretion, increased expression and structural rearrangement of tight junction proteins in the small bowel and colon with improved gut barrier function for translocation of luminal contents that results into improved health of humans suffering from inflammatory and metabolic diseases and disorders (Barbara et al., 2012).

5. Probiotics and antimicrobial effects against potential pathogens:

The antimicrobial activity of probiotics against potential enteric pathogens like Salmonella Typhimurium, Escherichia coli, Enterococcus faecalis, Staphylococcus aureus and Clostridium difficile, Klebsiella pneumonia and vaginal pathogens like Gardnerella vaginalis and Candida albicans has been extensively studied (Tejero-Sarinena et al., 2012). The main antimicrobial mechanisms include the production of organic acids such as lactic and acetic acids that consequently lowers the culture pH, Secretion of bacteriocins and antimicrobial peptides, production of H2O2 and nitric oxide besides metabolites like short chain fatty acids that are antagonistic to potential pathogens.

6. Mechanisms of probiotics action in the prevention and treatment of intestinal diseases

Changes in gut microbial composition with increased numbers of potentially pathogenic species is the main cause in the development of gastrointestinal disorders and infections like antibiotic-associated diarrhea and Clostridium difficile associated diarrhea, functional bowel problems (constipation and irritable bowel syndrome), inflammatory bowel diseases [Crohn's disease (CD) and ulcerative colitis (UC)] among people of various age groups (Malaguarnera et al., 2012). This dysbiosis in gut microbial composition alters the gut barrier function, intestinal immune system and inflammation that are responsible for development of above mentioned intestinal diseases. However, Probiotics have been found effective in the treatment of these diseases with improved gut microbial balance, enhanced intestinal barrier function, improved exclusion of pathogens from intestinal epithelium, modulation of immunity and improved bowel dysmotility (Hosseini et al., 2012).
7. Mechanisms of probiotics in the management of metabolic disorders

Probiotics have recently emerged as the prospective biotherapeutics in the management of metabolic syndrome with proven efficacy demonstrated in various in vitro and in vivo animal models adequately supported with their established multifunctional roles and mechanism of action for the prevention and disease treatment. The major mechanisms under this involves improved gut microbial balance with conjugated linoleic acid (CLA) production, decreased food intake, decreased abdominal adiposity and total cholesterol, decreased inflammatory tone with improved mucosal integrity in the management of metabolic syndrome (MetS) like obesity and type 2 diabetes (Mallappa et al., 2012).

8. Conclusion

The mechanisms of action of probiotics on human health are highly strain dependent, host specific and multi-faceted. The established mechanisms result in the development of probiotic product with specific health effect for the target population. However, further indepth studies with regard to specific beneficial effects of probiotics are required to be conducted at molecular level to understand the exact mechanisms of multiple novel health effects of probiotics in order to develop effective dietary therapies for the betterment of human health and wellbeing.

9. Suggested Reading


1. Introduction

In recent development consumers’ protection from food borne hazards has become a compelling duty for policy makers across the globe. The recent occurrence of serious food scares and food contamination events—such as *Salmonella* contagion in peanut butter, *E.coli* O104:H4 through seed sprouts in Germany, *Listeria monocytogenes* outbreak through melons in Colorado farm, USA, milk contamination with melamine in China, aerated drinks contamination with pesticide in India has raised food safety concerns and its impact on health, marketing and foreign trade. New serious chemical hazards have emerged in the food chain, such as natural toxicants like mycotoxins and marine toxins, environmental contaminants, such as mercury lead, and naturally occurring substances in plants. Although, traditional approaches have proved largely successful, risk assessment now also needs to take account of susceptible populations, combined with low level of exposure to several chemicals and effects on development of the fatal neural system.

Food borne diseases have a significant impact not only on health but also on development. Moreover, globalization of the food trade and development of international food standards have raised awareness of the interaction between food safety and export potential for developing countries. With India being a member of the CAC, the Ministry of Health and Family Welfare, has the primary responsibility for determination of Government policy relating to food standards and enforcement of food control including national position on various issues relating to Codex. With the global food industry looking towards India as a food hot-spot, it is about time the national food legislation is aligned with Codex, encouraging innovation and facilitating trade without compromising consumer safety. CAC is regarded as the ‘World Authority’ on food standards (Joint FAO/WHO Food Standard Programme). Codex’s focused objectives of (1) protecting consumers and (2) facilitating trade are shared by member countries and its standards based on scientific evidence and risk analysis principles are followed and/or adopted partially or in totality by countries around the world. The WTO in its Sanitary and Phytosanitary (SPS) Agreement recognizes the Codex standards as the global reference standards for consumers, food producers, processors, national food control agencies and all others involved in international food trade. The Agreement on the Application of SPS Measures and the Agreement on Technical Barriers to Trade (TBT) also encourage the international harmonization of food standards. Codex standards have thus become the benchmarks against which national food control measures and regulations are evaluated under the relevant provisions of the WTO Agreements.

The introduction of integrated food law provides the much required “one law—one–regulator” platform for raising the food safety standards of India to much global standards. Its speedy and effective implementation is quickly warranted to put India onto the global map. This would require an enabling implementation environment focused on creation of transparency, awareness creation, capacity building, certification of raw material & traceability system, developing right infrastructure, extensive R&D capacity and compliance of milk and milk products for FSSAI standards for Microbiological criteria for hygiene and safety indicators and presence of non-microbial contaminants:
antibiotic residues, aflatoxin M1, pesticides, heavy metals etc., so as to match the dynamically changing requirements of food safety and standards. The initiatives would also require a wide spread awareness and promotion campaign focused on changing the mindset of food producers so as to encourage adherence to food safety standards.

2. Setting public health goals

2.1 Concept of appropriate level of protection

During the past decade, there has been increased interest and effort in developing tools to more effectively link the requirements of food safety programs with their expected public health impact. An appropriate level of protection (ALOP), expressed in terms of a desired reduction in the current level of risk, could be defined as the food safety goal. An ALOP is currently defined as ‘a statement of the degree of public health protection that is to be achieved by the food safety system’. An explicit description of an ALOP may be in terms of the probability of an adverse public health consequence or the incidence of disease (e.g. the number of cases per 1, 00, 000 populations per year). Translation of an ALOP into a Food Safety Objective (FSO), expressed in terms of the required level of hazard control in food, provides a measurable target for producers, manufacturers and control authorities. An FSO is defined as ‘the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides an appropriate level of protection’. An alternative definition of an FSO might be a limit to the prevalence and the average concentration of a microbial hazard in food, at an appropriate step in the food chain at or near the point of consumption that provides the appropriate level of protection. This ALOP has also been called ‘acceptable level of risk’ (ALR). This term is similar to the expression ‘tolerable level of risk’ (TLR) preferred by the ICMSF, because it recognizes that risks related to the consumption of food are seldom accepted, but at best tolerated. One of the tasks of governmental risk managers is thus to decide upon what is adequate, appropriate or tolerable in terms of food safety or health risk. How they have to do this is not described in detail by the WTO or the Codex. However, the determination of ALOP/TLR should be science based, should include economic and social factors and should minimize negative trade effects. Integral to the agreement is that imported food should not compromise the ALOP. An exporting country can contest an importing country's judgment that a food is not meeting the ALOP by using scientific methods such as risk assessment, Codex standards, codes and guidelines. A country cannot demand that imported foods are ‘safer’ than similar domestically produced foods.

2.2 Concept of Risk Analysis

Risk analysis (RA) and its component parts (risk assessment, risk management and risk communication) should be used as a tool in evaluating and controlling microbiological hazards. A risk-management based approach (Fig. 1) is required to develop recommendations to ensure consumer protection and facilitate fair practices in the food trade.
Fig. 1. Principle of Risk assessment described by Codex Alimentarius Commission

This structured approach may employ microbiological risk assessment and may utilize a spectrum of risk communication products including guidance documents, codes of hygiene practice, food safety objectives (FSO) and microbiological criteria. Some general guidelines used to manage pathogens in foods have been described by ICMSF (2002), indicating the respective roles of industry and government. A series of steps is described, including:

a. analysis of epidemiological data which may give rise to concern for public health or a need for improved controls;
b. risk evaluation by an expert panel or through quantitative risk assessment;
c. establishment of an FSO when necessary;
d. assessing whether the FSO is technologically achievable through preliminary process and/or product formulation criteria and
e. if the FSO is achievable, establishment of process/product requirements.

2.3 Risk assessment
Risk assessment is the characterization and estimation of potential adverse health effects associated with exposure of individuals or populations to hazardous materials or situations. Risk assessment of microbiological hazards in foods has been identified as a priority area of work by the CAC. Risk assessment for microbiological hazards in foods is defined by the CAC as a scientifically based process consisting of four components: hazard identification, exposure assessment, hazard characterization, and risk characterization.

I. Hazard identification is predominantly a qualitative process intended to identify microorganisms or microbial toxins of concern in food or water. It can include information on the hazard of concern as well as relevant related data, such as clinical and surveillance data.

II. Exposure assessment should provide an estimate, with associated uncertainty, of the occurrence and level of the pathogen in a specified portion of food at the time of
consumption, or in a specified volume of water using a production-to-consumption approach. While a mean value may be used, more accurate estimates will include an estimate of the distribution of exposures. This will typically include identification of the annual food and water consumption frequencies and weights or volumes for a given population or subpopulations(s), and should combine the information to estimate the population exposure to pathogens through a certain food or water commodity.

III. **Hazard characterization** provides a description of the adverse health effects that may result from ingestion of a microorganism. When data are available, the hazard characterization should present quantitative information in terms of a dose-response relationship and the probability of adverse outcomes.

IV. **Risk characterization** is the integration of the three previous steps to obtain a risk estimate (i.e. an estimate of the likelihood and severity of the adverse health effects that would occur in a given population, with associated uncertainties).

The goal of a risk assessment may be to provide an estimate of the level of illness from a pathogen in a given population, but may also be limited to evaluation of one or several step(s) in a food production or processing system. When requesting a risk assessment, the risk manager should be specific with regard to the problem with which the risk manager needs to deal, the questions to be addressed by the risk assessment, and an indication of the measures the manager would consider or has available for the reduction of illness.

2.4 Risk management

The process, distinct from risk assessment, of weighing policy alternatives in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

2.5 Risk communication

The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

3. **Codex Alimentarius Commission**

The CAC is a body of United Nations (UN) established by FAO in 1961 and is an intergovernmental organisation that coordinates food standards at the international level. Its main objectives are to protect the health of consumers and ensure fair practices in food trade. The Codex Food Code (CFC) attempts to create harmonized standards. Prior to the SPS Agreement, the CFC could be adopted, applied and/ or ignored at the discretion of a government. However, the CFC has now been adopted within the SPS Agreement as the benchmark. The CAC has proved to be most successful in achieving international harmonization in food quality and safety requirements. It has formulated international standards for a wide range of food products and specific requirements covering pesticide residues, food additives, veterinary drug residues, hygiene, food contaminants, labelling, etc. These codex recommendations are used by governments to determine and refine policies and programmes under their national food control system. More recently, Codex has embarked on a series of activities based on risk assessment to address microbiological hazards in foods, an area previously unattended. Codex work has created worldwide
awareness of food safety, quality and consumer protection issues, and has achieved international consensus on how to deal with them scientifically, through a risk-based approach. As a result, there has been a continuous appraisal of the principles of food safety and quality at the international level. There is increasing pressure for the adoption of these principles at the national level. Quality assurance systems have become a focal point for inclusion in the work of Codex. As an example, the CAC has recently adopted guidelines for the application of the HACCP system. The HACCP approach, along with the use of GMPs, is strongly recognized and recommended by Codex. The principal consideration behind the development of any Codex standard, guideline or other recommendation is the protection of consumer’s health.

4. HACCP system

HACCP stands for Hazard Analysis Critical Control Point. HACCP is a systematic approach to the identification, evaluation, and control of food safety hazards. It is a proactive strategy where hazards are identified and assessed, and control measures are developed to prevent, reduce, or eliminate a hazard.

The HACCP system, which is science based and systematic, identifies specific hazards and measures for their control to ensure the safety of food. HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing. Any HACCP system is capable of accommodating change, such as advances in equipment design, processing procedures or technological developments.

HACCP can be applied throughout the food chain from primary production to final consumption and its implementation should be guided by scientific evidence of risks to human health. As well as enhancing food safety, implementation of HACCP can provide other significant benefits. In addition, the application of HACCP systems can aid inspection by regulatory authorities and promote international trade by increasing confidence in food safety. The successful application of HACCP requires the full commitment and involvement of management and the work force. It also requires a multidisciplinary approach; this multidisciplinary approach should include, when appropriate, expertise in agronomy, veterinary health, production, microbiology, medicine, public health, food technology, environmental health, chemistry and engineering, according to the particular study. The application of HACCP is compatible with the implementation of quality management systems, such as the ISO 9000 series, and is the system of choice in the management of food safety within such systems.

5. Management Systems for Quality and Food Safety

Excellence in food quality and safety has taken a tangible form with the advent of ISO 9000 Quality Management System and HACCP standards. ISO 9000 encompasses all the activities of a company to ensure that it meets its quality objectives, while HACCP is directed towards ensuring food safety. The ISO 9000 standards were brought by the International Organization for Standardization (ISO) and the HACCP standards by the CAC. These standards have assumed importance worldwide both as an essential requirement to tap the market potential and as a marketable feature of the company. Since the global market has become more demanding in terms of quality, safety and timely delivery, installation of the ISO 9000 Quality Management System and HACCP by the food industry is essential for getting a competitive international edge. Food Safety Programs may need to be implemented to meet regulatory requirements, retailer requirements or manufacturer’s requirements (Fig. 2).
6. ISO 9000 Quality Management Systems

The ISO 9000 system is looked at as a system with minimum quality requirements. It builds a baseline system for managing quality. The focus, therefore, is on designing a total quality management system, one that complies with external standards, but includes the specific requirement of industry and integrates elements of competitiveness.

Fig. 2 Process module of the standard ISO 22000:2005

7. Conclusion

The introduction of FSSA provides the much required “one law-one regulator” platform for raising the food safety standards of India to match global standards. Its speedy and effective implementation is quickly warranted to put India onto the global food map. This would require an enabling implementation environment focused on creation of transparency, developing right infrastructure and extensive R&D capacity so as to match the dynamically changing requirements of food safety and standards. The initiative would also require a wide spread awareness and promotion campaign focused on changing the mindset of food producers so as to encourage adherence to food safety standards.

8. Suggested Reading

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Validation of Health Claims of Functional Foods through Animal Assays

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1. Introduction

The relationship between food and health has long been known to exist. Today, the fundamental concept of food is changing from one that involves the maintenance of life to one that uses food to improve health and contribute to a better quality of life. As a result, there is great interest in this connection by public health officials, consumers, and the food sector industry. These foods once referred to as “health foods,” are now described as “functional foods”. Functional foods, designer foods, pharma foods and nutraceuticals are synonyms for the components of diet that possess unique disease preventing/curing ability. Epidemiological studies and randomized clinical trials carried out in different parts of the world have demonstrated or at least suggested numerous health effects related to functional foods consumption, such as reduction of cancer risk, improvement of heart health, enhancement of immune functions, improvement of gastrointestinal health, reduction of osteoporosis etc. There is no doubt that dairy products are functional foods. Nutritional significance of milk molecules is well documented but beyond the known nutrients i.e. proteins, vitamins, milk and milk constituents have clearly more to offer and scientist are scurrying to discovery exactly which milk components might fend off specific diseases.. They are one of the best sources of calcium; an essential nutrient which can prevent osteoporosis and possibly colon cancer. In addition to calcium, however, recent research has focused specifically on other components in dairy products viz. conjugated linoleic acid (CLA), butyric acid, lactoferrin, lactulose, galacto-oligosaccharides (GOS), bioactive peptides and probiotics. Basically functional dairy foods can be categorized as probiotic dairy foods, fortified milk products and nutraceuticals based dairy products like whey proteins and peptides etc. Ever-expanding arrays of previously unknown molecules are being uncovered. But there exact metabolic role and how these can be utilized in designer food, need to be elucidated. In a strict sense, health claims should be both implicit and explicit and scientific validation is increasingly the litmus test.

2. Scientific basis for identifying functional foods

The scientific evidence for functional foods and their bioactive components can be categorized into four distinct areas: Epidemiologic studies, In-vitro Laboratory studies, Animal Studies and Clinical trials. The first step in the research and development of a functional food is to identify a functional factor, condition or compound that produces a specific effect, which is potentially beneficial for health. Hence, it is necessary to investigate the interaction of this compound with other dietary elements and find out its function within the organism at different levels (genomic, molecular, cellular and physiological). One important aspect for functional ingredients is to evaluate the security margins of effective doses in order to produce the functional effects. These margins must be safe and applicable to all main population groups, including those who can be expected to consume them most. To cover this knowledge in depth requires basic fundamental research and the elucidation of the mechanism of action. Therefore, sound scientific evidence is required before a functional effect can be defined. Then, it must be proved in relevant animal models and in humans by means of well-designed
epidemiological and intervention studies (Bellisle, 2002). Identification and validation of good, quantifiable biomarkers, sensitive to dietary modulation and reflecting the step in the process between food intake and functional effects are of great help (Hasler et al, 2000).

3. Safety of functional foods

Prior to conducting efficacy trials, there is need to be satisfied that the intended dose level of the material will not present a health hazard to the recipients. Although the functional food may have been already part of the human diet somewhere in the world, to a degree, the intended "dose level" of the food product may be much higher than previously encountered and the clinician may want to be reassured of the absence of potential adverse effects.

The optimal levels of the majority of the biologically active components currently under investigation have yet to be determined. In addition, a number of animal studies show that some of the phytochemicals (e.g., allyl isothiocyanate) that have cancer-preventing properties have been shown to be carcinogenic at high concentrations (Ames et al., 1990). Thus, Paracelsus' 15th century doctrine that "All substances are poisons . . . the right dose differentiates a poison from a remedy" is even more pertinent today given the proclivity for dietary supplements. The benefits and risks to individuals and populations as a whole must be weighed carefully when considering the widespread use of physiologically-active functional foods. For example, what are the risks of recommending the increased intake of compounds (e.g., isoflavones) that may modulate estrogen metabolism? Soy phytoestrogens may represent a "double-edged sword" because of reports that genistein may actually promote certain types of tumors in animals (Rao et al., 1997). Knowledge of toxicity of functional food components is crucial to decrease the risk: benefit ratio.

Animal studies are a major element in the safety assessment of many compounds such as pesticides, pharmaceuticals, industrial chemicals and food additives. In most cases however, the test substance is well characterized, of known purity, of no particular nutritional value and human exposure is generally low. It is therefore relatively straightforward to feed such compounds to animals at a range of doses, some several orders of magnitude greater than the expected human exposure levels, in order to identify any potential adverse health effects of importance to humans. In this way it is possible, in most cases, to determine levels of exposure at which adverse effects are not observed, and so set safe upper limits by the application of appropriate safety factors. By contrast, foods are complex mixtures of compounds characterized by wide variation in composition and nutritional value. Due to their bulk and effect on satiety they can usually only be fed to animals at low multiples of the amounts that might be present in the human diet. In addition, a key factor to consider in conducting animal studies on foods is the nutritional value and balance of the diets used, to try to avoid the induction of adverse effects which are not related directly to the material itself. When assessing a food product for any toxic potential, the concept of ' wholesomeness' has to be considered. This term is applied to the combined assessment of toxicology and nutritional balance. Detecting any potential adverse effects and relating these conclusively to an individual characteristic of the food can therefore be extremely difficult. Another consideration in deciding the need for animal studies is whether it is appropriate to subject experimental animals to such a study if it is unlikely to give rise to meaningful information.

In a conventional toxicology study, when the test material is a pharmaceutical or pesticidal active ingredient, evidence of toxicity, such as retarded growth, usually occurs at a much lower treatment level than this. However, for food materials, a much higher
treatment level may be needed to achieve a similar effect. If high dose levels are to be used on animal feeding studies, then a thorough knowledge of the nutritional properties of the functional food (for example energy value, protein content and the bioavailability of micro nutrients) is essential, so that the test diets can be compensated for nutritional imbalance in order to achieve the normal healthy development of the animals. If the predicted usage levels of the functional food by consumers or clinical trial subjects is high, such that the safety margin generated by the animal feeding studies is low, then absorption and metabolism studies in animals followed eventually by similar studies in humans provide additional reassurance of the predictivity of the animal model.

4. Different Animal models and requirements for validation of functional foods

Depending upon the type of bioactive component and evaluation of its role in functional food, animal model are chosen. For carrying out cardiovascular health related studies, rats and rabbits are among the best model. For validation of immune response mice model is the best. Some of the important animal models generally used for validation are listed below

- Zebrafish for the evaluation of systemic effects of intestinal processes
- Conventional rodent models (rat, mice and guinea pigs)
- Germ-free and human-microbiota associated rodents for microbiota analysis and host-bacteria interactions
- Specific rodent models for health targets (e.g. obesity, immune-modulation, …)
- Higher animals (pigs, ruminants) to study local and systemic effects of intestinal processes (e.g. Immune-modulation, cholesterol, …)
- Protocol and design of the experiment
- Ethical approval
- Organization and recruitment
- Analysis of different targets
- Intestinal comfort, tolerance
- Modulation of microbial community
- Analysis of blood and serum (e.g. cholesterol, immune parameters, …)
- *Ex vivo* experiments (e.g. immune response of isolated monocytes)

5. Efficacy of functional foods

For functional food products, it is essential to substantiate the claimed health benefit. In addition to safety data, the need to show efficacy of the functional food may also necessitate safety testing. It is important to select an appropriate set of biomarkers to monitor the results of exposure. Markers must be scientifically well established and chosen to reflect accurately the processes of interest. Only then can the effect of consuming a functional food on a valid proxy for the final endpoint – i.e. an improved state of health and wellbeing or reduction in disease risk – be studied.

Markers could be chosen to reflect:

5.1 A key target biological function:

e.g. Bacterial populations in the gut can be measured to demonstrate that a probiotic has successfully passed through the stomach and could potentially have a beneficial effect in the lower GI tract;

e.g. antioxidants might be evaluated by measuring paraoxonase activity or total antioxidant scavenging capacity

e.g. a sterol/stanol cholesterol-lowering agent could be assessed by measuring circulating cholesterols (HDL/LDL ratio)
e.g. For Iron bioavailability studies hemoglobin, ferritin and transferrin levels are usually measured.

5.2 A key stage in the development of a disease
e.g. Bone mineral density can be used as a marker in the study of a functional food evaluating potential benefit in reducing the risk of osteoporosis
e.g. Flow mediated dilatation (FMD) can be used in the study of a food component designed to improve endothelial function and so reduce the risk of cardiovascular disease.

Development of nutritional biomarkers is a challenge. Many biomarkers have been proposed but relatively few have actually been established because of the complexity of disease mechanisms and the limited capability of a single biomarker to reflect the collective impact of multiple biochemical effects on clinical outcome. Whatever markers are used, they should be fully validated, easy to measure and reproducible in different centers. Care should be taken over their selection and the interpretation of results since they are likely to be influenced by the nutritional status of the individual and confounded by disease states or genetic polymorphisms.

6. Conclusion
Mounting evidence supports the observation that functional foods containing physiologically-active components, either from plant or animal sources, may enhance health. It should be stressed, however, that functional foods are not a magic bullet or universal panacea for poor health habits. There are no "good" or "bad" foods, but there are good or bad diets. Health-conscious consumers are increasingly seeking functional foods in an effort to control their own health and well-being. Claims about health benefits of functional foods must be based on sound scientific criteria. A number of factors complicate the establishment of a strong scientific foundation, however. These factors include the complexity of the food substance, effects on the food, compensatory metabolic changes that may occur with dietary changes, and, lack of surrogate markers of disease development. Additional research is necessary to substantiate the potential health benefits of those foods for which the diet-health relationships are not sufficiently scientifically validated.

7. Suggested Reading
Emerging and re-emerging diseases

Food borne diseases result from ingestion of a wide variety of foods contaminated with pathogenic microorganisms, microbial toxins, or chemicals. Food-borne disease outbreaks are defined as the occurrence of 2 or more cases of a similar illness resulting from ingestion of a common food or when observed number of cases of a particular disease exceeds the expected number. These can be confirmed (when at least one causal agent is identified) or suspected (based on clinical and epidemiological information). Although most cases are sporadic, these diseases draw attention due to outbreaks, investigation of which can help in initiating control measures. Emerging infectious diseases are “those causing illnesses that have only recently appeared or been recognized in a population or that are well recognized but are rapidly increasing in incidence or geographic range”. Approximately amongst 1,415 known species of microorganisms that produce disease in human beings 60% are zoonotic. Zoonotic foodborne diseases are those infections or disease that can be transmitted directly or indirectly between animal and humans. Approximately 175 pathogens are associated with diseases considered to be emerging. Since 1977, new or newly characterized foodborne pathogens have been recognized at the rate of approximately one every 2 years.

2. Food-borne illnesses

The global burden of infectious diarrhea involves 3-5 billion cases and nearly 1.8 million deaths annually, mainly in young children, caused by contaminated food and water. More than 200 known diseases are transmitted through food, and more than half of all recognized foodborne disease outbreaks have unknown causes, indicating the real number of disease-causing agents is likely much larger than 200. The symptoms of foodborne illnesses range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal syndromes. Foodborne illness poses a significant economic burden for nations, damages consumer confidence and impacts international trading of food products.

3. Incidence

As only a small proportion of illnesses are diagnosed and reported and also the periodic assessments of total episodes of illness are needed. Therefore the actual incidence of foodborne diseases is unknown. Only the estimates of the overall number of episodes of foodborne illness are used for allocating resources and prioritizing interventions. According to the CDC, estimated 76 million cases of food-borne disease are reported annually in the United States with approximately 5000 deaths. According to a recent report in US amongst 168 foodborne pathogens leading 14 pathogens represent over 95 percent of the annual illnesses and hospitalizations, and almost 98 percent of the deaths (Table 1). These 14 foodborne pathogens are estimated to cause 14.1 billion dollars (2009) in cost of illness, and loss of over 61,000 QALYs per year. WHO estimated worldwide, the number of cases of gastroenteritis associated with food is estimated to be between 68 million and 275 million per year (Naravaneni and Jamil 2005).

4. Why does food-borne disease emerge and remerge?
Substantial progress has been made in preventing food borne diseases. It was ruled in the pre-antibiotic era by disinfection of drinking water, sewage treatment, milk sanitation and pasteurization, and shellfish bed sanitation etc. Microorganisms are not static entities; they may cross the species barrier, gain virulence factors or increased toxic production lead to reemergence. The growing amount of antibiotic-resistant strains is an example. There are several reasons that change the pathogenic spectrum of foodborne pathogens over time. They include environmental adaptations by microorganism itself, globalization of food business introduce unfamiliar pathogens to new geographical areas and new food chain niche. The changes in eating habits and life style of consumers expose pathogen to new population. As a result, the frequency of specific infections occurrence can change substantially. Although several technologies are practiced to limit or prevent the contamination from occurring still several outbreaks are reported in developed and developing countries.

5. Food-borne pathogens of concern

According to Emerging pathogens Institute’s report published in 2011 more than 90 percent of this health burden in US is caused by five pathogens: Salmonella spp., Campylobacter spp., Listeria monocytogenes, Toxoplasma gondii and Norovirus. However, the data in developing countries not available may be due to the sporadic nature of outbreaks or lack of organized system to manage the outbreaks. Several foods regulatory entities the common microorganisms that cause food borne diseases are Salmonella, Pathogenic Escherichia coli, Campylobacter and Listeria, beside other, less common infectious species.

5.1 Salmonella spp.

Salmonella is the leading disease-causing bug overall, causing more than $3 billion in disease burden annually. In addition to poultry, Salmonella-contaminated produce, eggs and multi-ingredient foods all rank in the Top 10. Salmonella infections, even though they have been mostly associated with poultry have been also linked to outbreaks associated with the consumption of various other types of foods (Table 2). It has been reported that salmonellosis has been caused by less than 10 cells of Salmonella in Cheddar cheese. The infectious dose of this outbreak was relatively low (0.36-4.3 most probable number/100 g) indicating that low level contamination can cause Salmonella outbreaks that may be difficult to detect. The typical symptoms of the illness include nausea, vomiting, and diarrhea while additional complications associated with the infection include septicemia, or reactive arthritis.

5.2 L. monocytogenes

L. monocytogenes is the causative agent of listeriosis, a severe infectious foodborne disease, which is characterized by a very high fatality rate compared with those of other foodborne bacteria. Listeriosis preferentially affects individuals, whose immune system is perturbed, including pregnant women, newborns, old and immune compromised people, even though the level of contamination play also a major role for the pathogen to cause infection. Dairy products contaminated with L. monocytogenes have been implicated at almost the half of the reported listeriosis outbreaks (Table 3).

5.3 Shiga-toxin producing E. coli (STEC)

Hemorrhagic colitis and the hemolytic uremic syndrome (HUS) and E. coli O157:H7 is the most prominent serotype. E. coli O157:H7 is an emerging foodborne pathogen that
has caused more foodborne outbreaks related to consumption of raw and pasteurized milk compared to cheese. However, there are confirmed outbreaks with *E. coli* O157:H7 in cheese. The infective dose of *E coli* O157:H7 is 10-100 organisms, or even lower in the case of susceptible groups causing a wide range of clinical symptoms including non-bloody diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome, and death.

5.4 *Campylobacter* spp.

*Campylobacter* spp. are the most commonly reported cause of acute bacterial food poisoning in the European Union and, therefore, a major target for reduction in the public health burden of intestinal infectious diseases (Table 4). It has hospitalized more than 6,00,000 Americans at a cost of $1.3 billion per year. Infections with these microorganisms can cause acute illness such as vomiting but also can lead to hospitalization or death. Campylobacter infection can also cause paralysis and other neuromuscular problems. The report questions whether new safety standards announced by the USDA for young chickens and turkeys are sufficient, and recommends evaluating and tightening these standards over time.

6. **Antibiotic resistant bacteria**

Antimicrobial resistance is a particular issue for bacterial pathogens in the food chain. For more than 50 years, antimicrobial agents have been an essential component of infectious disease treatment, in both human and veterinary medicine, and the use of such agents has resulted in the development and spread of antimicrobial resistance. Any kind of antimicrobial use be it for human, animal or plant health purposes, can select for emergence of resistance and further promote the dissemination of resistant bacteria and resistance genes (Table 5). The public health consequences of this resistance can involve increased frequency of treatment failures and severity of infection including prolonged duration of illness, progression to systemic infections, increased hospitalization, and increased mortality. Food can be a source of both antimicrobial resistant bacteria and resistance genes. The presence in food of antimicrobial resistant pathogenic bacteria, such as *Salmonella* spp. or *Campylobacter* spp., can provide a direct infection hazard following ingestion or food handling. The transfer of resistance genes to a bacterium pathogenic for humans, either directly, or via a commensal, such as *E. coli* and *Enterococcus* spp., is viewed as an indirect hazard. Mobile genetic elements harboring resistance determinants can readily be transferred horizontally between bacteria from terrestrial animals, fish and humans and through various routes including food; furthermore, such transfer can take place in naturally occurring environments such as the kitchen. The relative contribution of each route to the risk of antimicrobial resistance in microorganisms of public health concern is too complex, with too many uncertainties to estimate, but there is increasing evidence that food is important. In addition, antimicrobial resistance may be a consequence of the direct contact of bacteria with the residues of antimicrobial agents in food. However, whilst acknowledging the toxicological concerns with antimicrobial residues, this mechanism of antimicrobial resistance spread seems less important than the previously mentioned routes. Of the 12 outbreaks related to dairy products, seven were associated with milk and five with cheese products. In ten of the outbreaks, the vehicle was described as unpasteurized or raw milk and/or cheese made from unpasteurized milk. Pasteurized milk was responsible for two of the dairy-related outbreaks, including one exceptionally large one. All outbreaks related to dairy products were caused by *Salmonella*-nine by *S. typhimurium*, two by *S. Newport*, and one by *S. Dublin*. Dairy-related outbreaks sickened at least 17,122, hospitalized 2,860, and killed 19 people (including the huge outbreak caused by *S. typhimurium*).
7. Conclusions

Food borne pathogens keep on emerging and re-emerging with changing human eating habits, increase in demand for all sorts of exotic food, increase in global market in vegetables and with increase in travel around the world now an epidemic in one country, if not properly controlled can spread to other countries within hours. Dynamic measures must be put in place to monitor such pathogens from farm to fork and from stable to table in order to ensure healthy food for the benefit of the human community. Early detection of food contamination at the farm level before the food goes to the market will ensure maximum protection of lives.

8. Selected Reading


<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Combined rank</th>
<th>Cost of illness (US $)</th>
<th>Illness (No.)</th>
<th>Hospitalization (No.)</th>
<th>Death (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>1</td>
<td>3,309</td>
<td>10,27,561</td>
<td>19,336</td>
<td>378</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>2</td>
<td>2,973</td>
<td>86,686</td>
<td>4,428</td>
<td>327</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>3</td>
<td>1,747</td>
<td>8,45,024</td>
<td>8,463</td>
<td>76</td>
</tr>
<tr>
<td><em>Listeria monocytogens</em></td>
<td>3</td>
<td>2,655</td>
<td>1,591</td>
<td>1,455</td>
<td>255</td>
</tr>
<tr>
<td><em>Norovirus</em></td>
<td>5</td>
<td>2,002</td>
<td>54,61,731</td>
<td>14,663</td>
<td>149</td>
</tr>
<tr>
<td><em>E.coli 0157:H7</em></td>
<td>6</td>
<td>272</td>
<td>63,153</td>
<td>2,138</td>
<td>20</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>6</td>
<td>309</td>
<td>9,65,958</td>
<td>438</td>
<td>26</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>8</td>
<td>252</td>
<td>97,656</td>
<td>533</td>
<td>29</td>
</tr>
<tr>
<td><em>Vibrio vulnificus</em></td>
<td>8</td>
<td>291</td>
<td>96</td>
<td>93</td>
<td>36</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>10</td>
<td>121</td>
<td>1,31,254</td>
<td>1,456</td>
<td>10</td>
</tr>
<tr>
<td><em>Vibrio</em> other</td>
<td>11</td>
<td>47</td>
<td>57,616</td>
<td>210</td>
<td>4</td>
</tr>
<tr>
<td><em>Cryptosporidium parvum</em></td>
<td>12</td>
<td>107</td>
<td>52,228</td>
<td>183</td>
<td>12</td>
</tr>
<tr>
<td><em>E.coli non -0157 STEC</em></td>
<td>13</td>
<td>26</td>
<td>1,12,752</td>
<td>271</td>
<td>0</td>
</tr>
<tr>
<td><em>Cyclospora cayetanensis</em></td>
<td>14</td>
<td>2</td>
<td>11,407</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>14,114</td>
<td>89,14,713</td>
<td>53,678</td>
<td>1,322</td>
</tr>
</tbody>
</table>

Table 2. Some important *Salmonella* outbreaks in the world

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Food</th>
<th>Serotype/Phage type</th>
<th>Cases (No.)</th>
<th>Deaths (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>Germany</td>
<td>Orange cream</td>
<td><em>S. enteritidis</em> PT4</td>
<td>109</td>
<td>4</td>
</tr>
</tbody>
</table>
### Table 3. Some important *L. monocytogenes* outbreaks in the world

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Food</th>
<th>Serotype</th>
<th>Cases (No.)</th>
<th>Deaths (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>USA</td>
<td>Chocolate Milk</td>
<td>-</td>
<td>54</td>
<td>-</td>
</tr>
<tr>
<td>1998</td>
<td>USA</td>
<td>Turkey products</td>
<td>4b</td>
<td>108</td>
<td>18</td>
</tr>
<tr>
<td>1998</td>
<td>Finland</td>
<td>Butter</td>
<td>3a</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>2000</td>
<td>France</td>
<td>Pork meat</td>
<td>4b</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>2000</td>
<td>USA</td>
<td>Turkey products</td>
<td>1/2a</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>2002</td>
<td>Canada</td>
<td>Raw milk cheese</td>
<td>-</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>2002</td>
<td>USA</td>
<td>Turkey products</td>
<td>-</td>
<td>54</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Sweden</td>
<td>Cheese</td>
<td>-</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>UK</td>
<td>Butter</td>
<td>-</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>Mexican cheese</td>
<td>4b</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Switzerland</td>
<td>Soft cheese</td>
<td>-</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Norway</td>
<td>Cheese</td>
<td>-</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Canada</td>
<td>Red meat</td>
<td>-</td>
<td>53</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>WA</td>
<td>Pasteurized milk</td>
<td>-</td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 4. Some Important *Campylobacter* Outbreaks in the World

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Food</th>
<th>Cases (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>UK &amp; Wales</td>
<td>Raw milk</td>
<td>333</td>
</tr>
<tr>
<td>2001-2002</td>
<td>Australia</td>
<td>Chicken</td>
<td>601</td>
</tr>
<tr>
<td>2005</td>
<td>Denmark</td>
<td>Chicken salad</td>
<td>4</td>
</tr>
<tr>
<td>2005</td>
<td>Scotland</td>
<td>Chicken pate</td>
<td>82</td>
</tr>
<tr>
<td>2005-2006</td>
<td>USA</td>
<td>Water</td>
<td>32</td>
</tr>
<tr>
<td>2007</td>
<td>USA</td>
<td>Cheese (from unpasteurized milk)</td>
<td>67</td>
</tr>
<tr>
<td>2007</td>
<td>Denmark</td>
<td>Water</td>
<td>16</td>
</tr>
</tbody>
</table>
### Table 5. Selected strains of antibiotic resistant micro-organisms

<table>
<thead>
<tr>
<th>Foodborne pathogen</th>
<th>Antibiotic resistances</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. Newport</em></td>
<td>Amoxicillin/clavulanic acid, sulfamethoxazole, ampicillin, cefoxitin, ceftiofur, chloramphenicol, cephalothin, streptomycin, tetracycline</td>
</tr>
<tr>
<td><em>S. typhimurium</em> DT104</td>
<td>Ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline</td>
</tr>
<tr>
<td><em>E. coli</em> O6:H16 (ETEC)</td>
<td>Ampicillin, chloramphenicol, nalidixic acid, streptomycin, sulfisoxazole, trimethoprim, sulfamethoxazole</td>
</tr>
<tr>
<td><em>S. aureus</em> (MRSA)</td>
<td>Methicillin</td>
</tr>
<tr>
<td><em>S. enteric</em> Serotype 4,5,12:i:-,</td>
<td>Ampicillin, streptomycin, sulfonamides, tetracycline, trimethoprim, sulfamethoxazole</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Ciprofloxacin, tetracycline</td>
</tr>
<tr>
<td><em>S. Heidelberg</em></td>
<td>Kanamycin, streptomycin, tetracycline</td>
</tr>
</tbody>
</table>
1. Introduction

In order to meet strict regulations on food safety issues and owing to greater public awareness of environmental contaminants there is a huge need to monitor wider range of food contaminants linked with supply chain so that quality and safe foods can be ensured to consumers. Analysts currently have a range of portable analytical techniques at their disposal for monitoring across a variety of contaminant namely pesticides, aflatoxin M1, drug residues, heavy metals and microbial pathogens in food matrixes. More recently, biosensors have emerged as another promising technology in the analyst's armoury, especially for applications requiring persistent monitoring. Biosensors are defined as analytical devices integrating biological elements and signal transducers. The biological elements such as enzymes, antibodies, receptors proteins, nucleic acids, cells, or tissue sections or bacterial spores interact specifically with an analyte, producing a signal that the transducer recognizes and converts into measurable parameters (Fig.1). The amount of signal generated is proportional to the concentration of the analyte, allowing for both quantitative and qualitative measurements in time. Although biosensors are of the essence for detection of contaminants but still operation of biosensors is a challenging task for their utility owing to the cost and shelf life of bio-recognition molecule. The resolution to above challenges is spore based biosensor which has evolved as robust, easy to use, simple, and inexpensive method for long term preservation, storage and transport of biosensing element.

![Fig. 1. Schematic diagram showing the main components of a biosensor](image)

The spore based biosensing systems are much superior in terms their activity, viability and analytical performance can be retained up to a period of 8 months when kept as dried spores at room temperature. The biosensors based on spore germination are real time sensing systems as germination process completes within minutes of sensing germinants in the environment. The spore production is a low priced process and its immobilization effortless process which curtails the cost of bio-recognition molecule employed in a biosensor.

2. Biosensor technology

The two main elements in a biosensor are a biological recognition element or bio-receptor and a signal transducer. The bio-receptor is a bio-molecule that recognizes the target analyte and can be divided into three distinct groups: bio-catalytic, bio-affinity, and microbe-based systems. Biocatalysis-based biosensors depend on the use of pure or crude
enzymes to moderate a biochemical reaction. For environmental applications, enzyme-based reactions involve enzymatic transformation of a pollutant or inhibition of enzyme activity by the pollutant. Enzyme inhibition approaches tend to cater for a larger number of environmental pollutants, usually of a particular chemical class such as antibiotic/drug residues, aflatoxin M1, pesticides and heavy metals in food system. However, such methods require the use of chromogen/or fluorogens for measuring the presence of target contaminants in food matrix. A spore inhibition based enzyme substrate assay (SIB-ESA) for detection of aflatoxins M1 milk has been developed. Spores of Bacillus spp. have been lyophilized/immobilized in micro centrifuge tube/sensor disk to which milk and substrate is added. In case where analyte is absent in milk system, specific indicator enzyme(s) are produced by active bio-sensing molecules which will act specifically on chromogenic/or fluorogenic substrate resulting in colored reaction (Kumar et al. 2010)/ or fluorescence as end product which is measured semi-quantitatively by either visually/ or using optical system at specific excitation/emission spectra.

3. Inhibition principle

Another system based on enzyme inhibition principle has been invented for monitoring of β-lactam antibiotics in milk. It is based on the principle of resistance mechanism of some β-lactamase generating Bacillus spp. Some spore forming bacteria such as B. cereus and B. licheniformis produce β-lactamase enzyme due to induction by β-lactam antibiotics and the enzyme production is proportional to the concentration of inducer present in milk. A real time microbial assay based on β-lactamase enzyme using starch iodine as colour indicator has been developed. The microbial assay is working on principle of non competitive enzyme action on inducer (β-lactam) resulting in indirect reduction of starch iodine mixture through penicilloic acid. A comparison of the intensity of the test reaction with that of a control was taken as criteria to determine whether the sample is positive or negative. The assay can detect specifically β- lactam groups in spiked milk within 15-20 min at regulatory codex limits with negligible sensitivity towards non β- lactam groups. The presence of Inhibitors other than antibiotic residues in milk did not interfere with the working principle of microbial assays (Kumar et al. 2009; Das et al. 2011; Kumar et al. 2012b; Gaare et al. 2012).

4. Affinity based biosensors

Bioaffinity-based biosensors rely on the use of proteins, DNA or microbial receptor to recognize and bind a particular target. For environmental applications such systems depend primarily on the use of antibodies. This is due to the ready availability of monoclonal and polyclonal antibodies directed toward a wide range of environmental pollutants, as well as the relative affinity and selectivity of these recognition proteins for a specific compound or closely related groups of compounds. Nucleic acid-based affinity and electrochemical biosensors for potential environmental applications have recently been reported. Application areas for these include the detection of chemically induced DNA damage and the detection of microorganisms through the hybridization of species-specific sequences of DNA. Charm assay (Charm Sciences Inc., USA) is an example of bio-affinity biosensor which employs an immune reaction to bind the antibiotic to a microbial receptor and detects this complex using a low-level ³H or ¹⁴C radio-label. The Charm assay can detect a family of antibiotics, notably β-lactams, sulphonamides, tetracyclines, novobiocin, aminoglycosides and macrolides, as well as various other substances such as chloramphenicol. The Charm II assay is an immune receptor test but is suitable for large laboratories only, requiring a range of laboratory equipment, including a
centrifuge and sample mixers to prepare samples as well as a scintillation counter to detect the radio-label.

5. Microbial biosensors

Microbial biosensors involves application of microorganisms as such/ or their spores as biological recognition element. These generally involve the measurement of microbial respiration, or its inhibition, by the analyte of interest. Compared to enzyme-based approaches, microorganism-based biosensors are relatively inexpensive to construct and can operate over a wide range of pH and temperature. The broad specificity of microbial biosensors to environmental toxins make them particularly applicable for general toxicity screening like biological oxygen demand (BOD) or in situations where the toxic compounds are well defined, or where there is a desire of measure total toxicity through a common mode of action.

A signal transducer is the second essential component of a biosensor. It converts the recognition event into a measurable signal. The transducer can take many forms depending upon the parameters being measured. The most well developed classes of transducers are potentiometric, amperometric, conductometric, optical, acoustic or piezoelectric etc. These utilize various electrochemical responses to measure changes in the electrical properties of the biological recognition element. Most of the reported potentiometric biosensors for detection of environmental pollutants have used enzymes that catalyze the consumption or production of protons. Phosphoric and carbamate pesticides can be evaluated through the use of a pH electrode that measures the activity of acetyl cholinesterase. The activity of the enzyme is affected by the presence of pesticides.

Further application of spore as signal transducer application targets real time detection of bacterial contamination using the inhibition of enzyme acetyl esterase coupled to spore germination using optical device for measurement. The use of spore as signal transducer is feasible if an illustrative knowledge of spore germination process and germinants are required. It involves selective enrichment of target bacteria in a selective media. The enriched bacterial cells will produce specific marker enzymes which act on germinogenic substrate and produce specific germinant (sugars and amino acids). The germinants induce spore germination and germination mediated concomitant de novo acetyl esterase enzymatic activity. As a consequence germination derived product can be easily detected by quantification of fluorescent signal produced as result of DAF hydrolysis by acetyl esterase. Based on above principle of germinogenic substrate detection of enterococci detection system has been developed which targets specific marker enzyme β-D glucosidase of enterococci with and aesculin as germinogenic substrate which releases germinant β-D glucose. The sensitivity of spore based bioassay was 5.66 log counts of cells in 5-6 hrs in spiked milk.

6. Optical biosensors

In the field of biosensors transducers based on optical detection techniques are also emerging. These may employ linear optical phenomenon, including fluorescence, phosphorescence, polarization, rotation, interference, surface plasmon resonance (SPR), total internal reflection fluorescence (TIRF), etc. or non-linear phenomena, such as second harmonic generation. Advantages of optical techniques involve the speed and reproducibility of the measurement. Microbial spore germination based optical biosensor for the detection of enterococci in milk is being developed in our laboratory (Fig. 2). The detection technique being used is electron multiple charged couple device (EMCCD), as
optical transducer which improvises the sensitivity as it equipped to detect germination of single spore (Kumar et al. 2012a).

![Operating Principle](image)

**Fig. 2. Principle of Microbial spore germination based optical biosensor for the detection of bacterial contaminants**

The basic requirement of a biosensor is that the biological material should bring the physico-chemical changes in close proximity of a transducer. In this direction immobilization technology has played a major role. Immobilization not only helps in forming the required close proximity between the biomaterial and the transducer, but also helps in stabilizing it for reuse. The biological material is immobilized directly on the transducer or in most cases, in membranes, which can subsequently be mounted on the transducer. Selection of a technique and/or support would depend on the nature of the biomaterial and the substrate and configuration of the transducer used.

7. **Immobilization techniques**

Some of the widely used immobilization techniques include adsorption, entrapment, covalent binding and cross-linking. Immobilization of enzymes and whole cells through adsorption perhaps is the simplest of all the techniques and was achieved successfully in monitoring of aflatoxin M₁ and enterococci on sensor disc/ or biochip using EMCCD system and plate reader. Most of these techniques have the drawbacks of weak adhesion as well as complexity of the process. Novel techniques have been developed for immobilizing viable or non-viable cells through adhesion on a variety of polymeric surfaces including glass, cotton fabric and synthetic polymeric membranes using poly-ethyl-enimine (PEI). This technique is gaining importance in the introduction of enzymes and microbes on transducer surfaces.

8. **Commercial biosensors**

Although most biosensors systems have been tested only on non-real samples (such as in distilled water or buffer solutions), a few biosensors applied to real samples have appeared in recent years. Some representative examples of their application to the determination of different classes of key pollutants and environmental quality parameters, such as biological oxygen demand (BOD), toxicity or endocrine effects, in a variety of matrices are listed in Table 1. The application of biosensors to real samples must be a necessary step before their commercialization, which is, in general, the aim of the device development. Results must also be validated by comparison with those obtained with standard protocols in order to get the acceptance of end users. Most commercial biosensors developed are focused in clinical applications, such as for glucose and lactate.
Prospective biosensor market for food, pharmaceutical, agriculture, military, veterinary and environment are still to be explored.

Table 1: Biosensors applied to the determination of pollutants in real samples

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sample source</th>
<th>Transducer, recognition element</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pesticides</td>
<td>River water</td>
<td>Optical, immunochemical</td>
</tr>
<tr>
<td>Phenols</td>
<td>Wastewater</td>
<td>Electrochemical, enzymatic</td>
</tr>
<tr>
<td>Linear alkyl benzene sulphonate (LAS)</td>
<td>River water</td>
<td>Electrochemical, bacteria</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Wastewater</td>
<td>Electrochemical, bacteria</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Wastewater</td>
<td>Optical, bacteria</td>
</tr>
<tr>
<td>Alkanes</td>
<td>Groundwater</td>
<td>Optical, bacteria</td>
</tr>
<tr>
<td>Estrogens and Xenoestrogens</td>
<td>Real water samples (lake and a sewage plant)</td>
<td>Optical, human estrogen receptor (EC)</td>
</tr>
<tr>
<td>BOD</td>
<td>River water</td>
<td>Optical, Pseudomonas sp.</td>
</tr>
<tr>
<td>Zinc dichromate chromate</td>
<td>Soil (extract)</td>
<td>Optical, bacteria</td>
</tr>
<tr>
<td>Mercury</td>
<td>Soil (extract)</td>
<td>Optical, Pseudomonas sp.</td>
</tr>
<tr>
<td>Arsenite Daunomycin, PCBs, aflatoxin</td>
<td>River water (pre-concentrated)</td>
<td>Electrochemical, DNA</td>
</tr>
<tr>
<td>Chlamydia trachomatis (DNA)</td>
<td>River water (pre-concentrated)</td>
<td>Electrochemical, DNA</td>
</tr>
<tr>
<td>Aflatoxin M1</td>
<td>Milk</td>
<td>Optical, Spore</td>
</tr>
<tr>
<td>Antibiotic (Broad Spectrum)</td>
<td>Milk</td>
<td>Optical, Spore</td>
</tr>
<tr>
<td>Antibiotic (β-lactam)</td>
<td>Milk</td>
<td>Optical, Spore</td>
</tr>
</tbody>
</table>

9. Enzyme based assay for Enterococci & Listeria monocytogenes

9.1 Development of Enzyme Substrate Assay (ESA) for Monitoring Enterococci in Milk

An Enzyme Substrate Assay (ESA) based on β-D-glucosidase activity was attempted for specific detection of Enterococci to meet the emerging demand of dairy industry. Four enrichment broths commercially available in the market were screened for selective recovery of Enterococci based on β-D-glucosidase activity. One of these broths namely Chromocult Enterococcus Broth (CEB) showed better performance in terms of selectivity and enzyme activity with partial inhibition of contaminants other than Enterococci.

The selected medium was further improved for desired features by increasing the concentration of sodium azide from 0.06 to 0.15g/100ml resulting in significant inhibitory effect on growth pattern of L. lactis, L. casei, Leuconostoc mesenteroides and L. monocytogenes. Other media components and supplements were also optimized for enhanced sensitivity and selectivity of Enterococcus sp. The optimized selective enrichment medium i.e. Esculin Based Sodium Azide Medium (EBSAM) demonstrated superior features in terms of sensitivity, selectivity, fastness, accuracy etc. and may be a suitable substitute for existing media used for routine monitoring of Enterococci in R&D institutions. Developed assay was screened for Enterococci count with 32 samples of raw milk and it could detect 2.67, 3.50, 4.25 and 4.8 log counts within incubation period of
12, 7½, 6½ and 5 hr respectively (Fig 3a & 3b). ESA could also detect Enterococci log counts of 2.84 in pasteurized milk within 12 hrs of incubation; however, assay was insensitive at very low level of 1.13 and 0.915 log counts. As such ESA developed in current investigation may find industrial application as Hygiene Indicator test for detection of Enterococci in raw milk & pasteurized milk within 5-12 hrs as against 36-48hrs required in conventional method (Thakur et al., 2010).

Fig 3a. Novel Microtechniques for detection of Enterococci

Fig 3b. Selectivity of EBSAM

9.1.1 Novel Features
- Cost effective (0.97/- per test)
- Better sensitivity
- Consistency in colour development within 5-12 hours
- Lab Validation with conventional method (ISO 7899-2:1984)
- Wide spectrum of application for raw, pasteurized, dried milk
- The test can be applied for assessing the hygiene status of equipment/utensils, air, water, personnel and plant environment
- Patent has been filed and a non-exclusive license cost of the technology is ~ 3.5 lakhs.

9.2 A Novel enzyme-substrate based bio-assay for real time detection of Listeria monocytogenes in milk system

This technique is based on the principle of targeting “enzyme-substrate reaction using chromogenic substrate(s) for specific unique marker enzyme(s) of target bacteria to releases free chromogen that can be visually detected by colour change /or by calorimetrically activity after initial enrichment of the bacterium in novel selective medium” (Fig. 4a & b).

9.2.1 Novel Features
- Cost effective (91.01/- per test)
- Better sensitivity
- Consistency in colour development within 5-24 hours
- Wide spectrum of application for raw, pasteurized, dried milk
• The test can be applied for assessing the hygiene status of equipment/utensils, water, personnel and plant environment
• Patent has been filed and a non-exclusive license cost of the technology is ~ 3.5 lakhs.

10. Future prospects
The hurdles to application of biosensors include:
• Diversity and complexity of samples.
• Relatively high development costs for single analyte systems,
• Limited shelf and operational life

Nevertheless, there are a number of areas where the unique capabilities of biosensors might be exploited to meet the requirements of environmental monitoring. Advances in areas such as multi-pollutant-screening could allow these techniques to be more competitive. The present scenario demands for increased range of detectable analytes with portable device structure. Solving the resulting integration issues will require further convergence with associated technologies such as biochemistry, polymer chemistry, electronics, micro-fluidics and separation technology. Micro-electro-mechanical systems or MEMS technology is one of the promising areas that may be going to fulfil these demands in future. The technology is an integration of mechanical elements, sensors, actuators, and electronics on a common silicon substrate through micro fabrication technology. Biochips and sensor arrays for detection of a wide range of hazardous chemical and biological agents can be made out of these MEMS based devices, making it feasible for simultaneous detection of multiple analytes. This also brings the lab-on-chip concept. However, Immobilization and stabilization of bio-molecules on these nano-devices may be a greater challenge. Some of the works in these areas have already been initiated. Utilization of molecular recognition ability of bio-molecules like avidin-biotin or streptavidin-biotin in conjunction with a lithographic technique is being investigated for the micro immobilization of enzymes on silicon wafers for biosensor applications.
Immobilization of enzymes on silicon supports has attracted attention in biosensor chip technology and a variety of classical techniques have been proposed.

There are interesting possibilities within the field of biosensors. Given the existing advances in biological sciences, coupled with advances in various other scientific and engineering disciplines, it is imminent that many analytical applications will be replaced by biosensors. A fruitful fusion between biological sciences and other disciplines will help to realize the full potential of this technology in the future.

11. Suggested Reading
Bacteriocins Produced by Lactic Acid Bacteria and their Application in Food System

Dr. R.K. Malik, Neha Pandey and Chhaya Goyal
Dairy Microbiology Division

1. Introduction

There has been scientific recognition of an essential need to control detrimental microorganisms in our environment, ever since the era of Louis Pasteur and Robert Koch. The discovery of penicillin by Alexander Fleming in 1929 opened the door for use of therapeutic antibiotics by the medical and veterinary communities to combat specific disease-causing organisms. However, in recent years, bacterial antibiotic resistance has been considered a problem due to the extensive use of classical antibiotics in treatment of human and animal diseases (Roy, 1997; Lipsitch et al., 2000; Yoneyama and Katsumata, 2006). As a consequence, multiple resistant strains appeared and spread causing difficulties and the restricted use of antibiotics as growth promoters. So, the continue development of new classes of antimicrobial agents has become of increasing importance for medicine and food products (Kumar and Schweiser, 2005; Fisher et al., 2005). In order to control the abusive use of antibiotics in food and feed products, one plausible alternative is the application of some bacterial peptides as antimicrobial substances in place of antibiotics of human application. Among them, bacteriocins produced by lactic acid bacteria have attracted increasing attention, since they are active in a nanomolar range and have no toxicity.

2. Bacteriocins

Bacteriocins stand out among the wide variety of antimicrobial ribosomal peptides synthesized by bacteria. They have been found in all major lineages of bacteria (Riley et al., 2002). Also it has been observed that some members of the Archaea also produce antimicrobial proteins resembling bacteriocins (O’Connor et al., 2002). Bacteriocins were first discovered by Andre Gratia and his workgroup in 1925 as ‘principle V’ (produced by a strain of Escherichia coli against another culture), however, the term “bacteriocin” was coined in 1953 (Jacob et al., 1953). These molecules are defined as ribosomally synthesized antimicrobial peptides, proteins or proteinaceous complexes produced by bacteria that act mainly against closely related species.

2.1 Bacteriocins produced by Gram negative bacteria

Bacteriocins produced by Gram-negative bacteria, are large proteins (>20 KDa). They can be divided into two groups, namely the colicins and the microcins. Colicins are large (25- to 80-KDa) bactericidal proteins produced by and toxic to some strains of Escherichia coli. Since their discovery the colicins have been the most extensively studied Gram negative bacteriocins, and they now serve as a model system for investigating the mechanisms of bacteriocin structure/function, genetic organization, ecology and evolution (Cascales et al., 2007). Colicins are shown to kill their targets by either membrane permeabilization or nucleic acid degradation (Braun et al., 1994; Riley and Wertz 2002; Smarda and Smajs 1998). Microcins are antimicrobial peptides also produced by members of the family Enterobacteriaceae (E.coli strains) under conditions of nutrient depletion that target microbes phylogenetically related to the producer strain. These are smaller (<10 KDa) in size. (Kolter et al.,1992). Microcins share more
properties with the bacteriocins produced by Gram positive bacteria, including thermostability, resistance to some proteases, relative hydrophobicity and resistance to extreme pH.

2.2 Bacteriocins produced by Gram positive bacteria

The bacteriocins produced by Gram positive bacteria are abundant and more diverse than those found in Gram negative bacteria. They are generally cationic, amphipathic small molecules that range in size from 2-6 kDa (> 8 k Da). These are often membrane permealizing peptides resembling the antimicrobial peptides produced by eukaryotes. They vary a lot in make-up and general structure. They have features in common such as heat stability, protease resistance and the fact that most run faster through an SDS-PAGE gel than predicted on the basis of their sizes. Available 3D-structures of some bacteriocins show that some of them are tightly folded peptides. Internal cross-linking supports the stability of many of these peptides. They differ from bacteriocins of Gram negative bacteria in two fundamental ways. First the bacteriocins produced by Gram positive bacteria are not necessarily lethal to the producing cell. Second, the Gram positive bacteria have evolved bacteriocin specific regulation, whereas bacteriocins of Gram negative bacteria rely solely on host regulatory networks (Braun et al., 1994; Smarda and Smajs 1998).

2.3 Bacteriocins produced by lactic acid bacteria

Lactic acid bacteria (LAB) constitute a diverse group of Gram-positive bacteria, characterized by some common morphological, metabolic and physiological traits. They are anaerobic bacteria, non-sporeulating, acid tolerant and produce mainly lactic acid as an end product of carbohydrate fermentation. Lactic acid bacteria consist of a number of diverse genera which include both homofermenters and heterofermenters based on the end product of their fermentation. The homofermenters produce lactic acid as the major product of fermentation of glucose and include the genera Lactococcus, Streptococcus and Pediococcus. In contrast, the heterofermenters produce a number of products besides lactic acid, such as carbon dioxide, acetic acid, and ethanol from the fermentation of glucose and includes the genus Leuconostoc and a subgroup of the genus Lactobacillus, the Betabacteria (Kandler et al., 1986).

The core LAB genera Lactobacillus, Lactococcus, Leuconostoc, Pediococcus and Streptococcus share a long history of safe usage in the processing of fermented foods. The antimicrobial effects and safety of LAB in food preservation is widely accepted (EFSA, 2005; de Vuyst and Frederic 2007). Their preservative effect is mainly due to the production of lactic acid and other organic acids which results in lowering of pH (Daeschel 1989). Preservation is enhanced by the production of other antimicrobial compounds, including hydrogen peroxide, CO2, diacetyl, acetaldehyde, and bacteriocins (Stiles and Hastings 1991; Klaenhammer 1988; 1993).

Lactic acid bacteria are capable of producing a wide range of ribosomally synthesized proteins and peptides which have antimicrobial activity to compete with other bacteria of the same species (narrow spectrum) or to counteract bacteria of other genera (broad spectrum) (Cotter et al., 2005). Since the initial discovery of bacteriocins in 1925, numerous bacteriocins have been isolated and characterized from lactic acid bacteria and some have acquired a status as potential antimicrobial agents because of their potential as food preservatives and antagonistic affect against important pathogens. The important ones are nisin, diplococcin, acidophilin, bulgarican, helveticins, lactacins, plantaricins and gassericins.
Table 1: Properties of some well characterized bacteriocins from LAB

<table>
<thead>
<tr>
<th>Bacteriocin</th>
<th>Producer organism</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin</td>
<td><em>Lactococcus lactis subsp.lactis</em> ATCC 11454</td>
<td>Lantibiotic, broad spectrum, chromosome / plasmid mediated, bactericidal, produced late in the growth cycle</td>
</tr>
<tr>
<td>Pediocin A</td>
<td><em>Pediococcus Pentosaceus</em> FBB61 and L-7230</td>
<td>Broad spectrum, plasmid mediated</td>
</tr>
<tr>
<td>Pediocin AcH</td>
<td><em>Pediococcus acidilactici</em> H</td>
<td>Broad spectrum, plasmid mediated</td>
</tr>
<tr>
<td>Pediocin 34</td>
<td><em>Pediococcus pentosaceous</em></td>
<td>Broad spectrum, chromosome mediated</td>
</tr>
<tr>
<td>Enterocin 99</td>
<td><em>Enterococcus faecium</em> 99</td>
<td>Broad spectrum, bactericidal</td>
</tr>
<tr>
<td>Leucocin</td>
<td><em>Leuconostoc gelidum</em> UAL 187</td>
<td>Broad spectrum, plasmid Mediated, bacteriostatic, produced early in the growth cycle</td>
</tr>
<tr>
<td>Helveticin J</td>
<td><em>Lactobacillus helveticus</em> 481</td>
<td>Narrow spectrum, chromosomally mediated, bactericidal</td>
</tr>
<tr>
<td>Carnobacteriocin</td>
<td><em>Carnobacterium piscicola</em> LV17</td>
<td>Narrow spectrum, plasmid mediated, produced early in the growth cycle.</td>
</tr>
<tr>
<td>Gassericin A</td>
<td><em>Lactobacillus gasseri</em> LA 39</td>
<td>Broad spectrum, plasmid mediated, bactericidal, circular peptide, heat stable</td>
</tr>
</tbody>
</table>

3. Classification of Bacteriocins

Bacteriocins produced by lactic acid bacteria, which have a long history of use in fermentation and meat and milk products preservation are the best characterized bacteriocins. The classification of bacteriocins, however, remains controversial. Klaenhammer classified bacteriocins into four classes (Klaenhammer 1993)- the lantibiotics, the unmodified peptides, the large heat labile bacteriolysins and the complex bacteriocins. By contrast, Cotter proposed two major divisions for bacteriocins (Cotter et al., 2005): Class I -the lantibiotics and Class II- the non-lantibiotic bacteriocins. Klaenhammer's classes IIc and IV were withdrawn in Cotter’s scheme since they were unproven entities. The novel post-translationally modified cyclic peptides were relegated to Class IIc. The class III heat labile bacteriolysins proposed by Klaenhammer exhibited all the defining characteristics of bacteriocins (bacteriolytic proteins such as lysostaphin and zoocin A), including genes encoding specific immunity factors. However, it was observed that some class III bacteriocins such as helveticin J do not kill by lytic mechanism and hence cannot be classified as bacteriolysins. Ness et al., (2007), grouped bacteriocins into two major classes, class I: lantibiotics; and class II: the heat-stable nonlantibiotics. In his classification scheme, they subdivided class II into four subclasses: IIa (antilisteria pediocin-like bacteriocins), IIb (two-peptide bacteriocins), IIc (leaderless peptidebacteriocins) and class IId (circular bacteriocins). Continuous discovery of new
bacteriocins with novel features requires an updating of the existing classification of bacteriocins.

4. Food Applications

Consumers have been consistently concerned about possible adverse health effects from the presence of chemical additives in their foods. As a result, consumers are drawn to natural and “fresher” foods with no chemical preservatives added. This perception, coupled with the increasing demand for minimally processed foods with long shelf life and convenience, has stimulated research interest in finding natural but effective preservatives. Bacteriocins, produced by LAB, may be considered natural preservatives or biopreservatives that fulfill these requirements. Biopreservation refers to the use of antagonistic microorganisms or their metabolic products to inhibit or destroy undesired microorganisms in foods to enhance food safety and extend shelf life (Schillinger et al., 1996). Three approaches are commonly used in the application of bacteriocins for biopreservation of foods (Schillinger et al., 1996):

(1) Inoculation of food with LAB that produce bacteriocin in the products. The ability of the LAB to grow and produce bacteriocin in the products is crucial for its successful use.

(2) Addition of purified or semi-purified bacteriocins as food preservatives.

(3) Use of a product previously fermented with a bacteriocin-producing strain as an ingredient in food processing.

4.1 Biopreservation of meat products

*L. monocytogenes* is a gram-positive, non-spore-forming facultatively anaerobic rod widely distributed in the natural environment. It can grow over a pH range of 4.1 to 9.6 and a temperature range of 0 to 45 °C. Moreover, it is relatively resistant to desiccation and can grow at *a*<sub>w</sub> values as low as 0.90. The ubiquitous nature of *L. monocytogenes*, its hardiness and ability to grow at refrigeration temperatures and anaerobic conditions make it a threat to the safety of foods. It is regarded as a major food safety problem because it can cause serious illnesses and death. The United States government has the most rigid policy regarding *L. monocytogenes* and set a zero tolerance level for *L. monocytogenes* in ready-to-eat foods (Jay 1996; Ryser and Marth 1999). It has been detected in a variety of foods and implicated in several foodborne outbreaks, such as turkey franks (Jay 1996). Many studies have been carried out to control *L. monocytogenes* in meat products since it is common within slaughterhouse and meat-packing environments and has been isolated from raw meat and cooked ready-to-eat meat products (Ryser and Marth 1999).

The activity of nisin alone at concentrations of 400 and 800 IU/g and in combination with 2% sodium chloride against *L. monocytogenes* in minced raw buffalo meat was examined by Pawaret *et al.* (2000). Murray and Richard (1997) evaluated the antilisterial activity of nisin A and pediocin AcH in decontamination of artificially contaminated pieces of raw pork. Nisin A was considerably more efficient than pediocin AcH, but after 2 d of storage, surviving bacteria in meat treated with each bacteriocin resumed growth at a rate similar to that of the control. Moreover, nisin was more stable than pediocin AcH. The loss of effectiveness, especially of pediocin AcH, was attributed to rapid degradation by meat proteases. In addition to nisin and pediocin, other LAB bacteriocins have been examined to control the growth of *Listeria*. Degnan *et al.* (1992) demonstrated the possibility of using bacteriocinogenic cultures of *Pediococcus acidilactici* (pediocin AcH producer) to control *L. monocytogenes* growth in vacuum-packaged all-beef wiener. When wiener was surface inoculated with *L. monocytogenes* and *P. acidilactici* and vacuum-packaged, *L. monocytogenes* and the *pediococci* survived in packages held at 4 °C, but the
Pediococci did not produce acid or pediocin during refrigerated storage. The primary reasons that nitrite is commonly used in curing meats is to stabilize red meat color and inhibit food spoilage and poisoning organisms, such as C. botulinum; however, nitrite can react with secondary amines in meats to form carcinogenic nitrosamines. This possible adverse health effect has prompted researchers to explore the potential of using bacteriocins as an alternative to nitrite. Rayman et al., (1981) reported that a combination of 3000 IU/g of nisin and 40 ppm of nitrite almost completely inhibited outgrowth of Clostridium sporogenes spores in meat slurries at 37 °C for 56 d; however, in a later study (Rayman et al., 1983) they found that up to 22000 IU/g of nisin in combination with 60 ppm of nitrite failed to prevent outgrowth of C. botulinum spores in meat slurries at pH 5.8. Reducing the pH was found to enhance nisin activity.

4.2 Biopreservation of dairy products

L. monocytogenes has been the documented cause of a number of outbreaks associated with dairy products, such as pasteurized milk (Fleming et al., 1985) and cheese (James et al., 1985), and nisin has been shown effective against L. monocytogenes in dairy products. Ferreira and Lund (1996) found that following inoculation of a nisin-resistant strain into long-life cottage cheese at pH 4.6 to 4.7, the number of L. monocytogenes decreased approximately 1-log10 cycle during storage at 20 °C for 7 d. Addition of nisin (2000 IU/g) to the cottage cheese increased the rate of inactivation to approximately 3-log10 cycles in 3 d. Davies et al., (1997) determined the efficacy of nisin to control L. monocytogenes in ricotta-type cheeses over 70 d at 6 to 8 °C. Addition of nisin (100 IU/ml) effectively inhibited the growth of L. monocytogenes for a period of 8 wk or more (dependent on cheese type). The control cheese contained unsafe levels of the organism within 1 to 2 wk of storage. Zottola et al., (1994) used nisin-containing cheddar cheese that had been made with nisin-producing lactococci as an ingredient in pasteurized process cheese or cold pack cheese spreads. The shelf life of the nisin-containing pasteurized process cheese (301 and 387 IU nisin/g) was significantly greater than that of the control cheese spreads. In cold pack cheese spreads, nisin (100 and 300 IU/g) significantly reduced the numbers of L. monocytogenes, S. aureus, and heat-shocked spores of C. sporogenes. Another problem in cheese production is the Clostridium-associated butyric acid fermentation. Nisin is commonly added to pasteurized processed cheese spreads to prevent the outgrowth of clostridia spores, such as Clostridium tyrobutyricum (Schillinger et al., 1996). One application of lacticin 3147, a broad-spectrum, 2-component bacteriocin produced by L. lactis subsp. lactis DPC 3147, is to control cheddar cheese quality by reducing non-starter LAB populations during ripening (Ross et al., 1999). Cheese manufactured with the lacticin 3147-producing transconjugant, L. lactis DPC4275, contained 2 log10 less non-starter LAB than control cheese after 6 mo of ripening. Moreover, cheese manufactured with 3 natural lacticin 3147-producing strains had no detectable non-starter LAB over the same time period. In cottage cheese the population of L. monocytogenes was reduced by 3-log10 cycles over a 1-wk ripening period when it was manufactured with L. lactis DPC4275; however, the number of Listeria in the control cheese, manufactured with a non-lacticin 3147-producing starter, remained unchanged (10^4 CFU/g). The lacticin 3147-producing transconjugant has also been used as a protective culture to inhibit Listeria on the surface of a mold-ripened cheese. Presence of the lacticin 3147 producer on the cheese surface reduced the number of L. monocytogenes by 3-log10 cycles (Ross et al., 1999).
4.3 Biopreservation of seafood products

The effectiveness of bacteriocins and protective cultures to control growth of \textit{L. monocytogenes} in vacuum-packed coldsmoked salmon has been demonstrated by several researchers. Katla et al., (2001) examined the inhibitory effect of sakacin\textsubscript{P} and/or \textit{L. sake} cultures (sakacin\textsubscript{P} producer) against \textit{L. monocytogenes} in cold-smoked salmon. The inhibitory effect of nisin in combination with carbon dioxide and low temperature on the survival of \textit{L. monocytogenes} incold-smoked salmon has also been investigated (Nilsson et al., 1997). Addition of nisin (500 or 1000 IU/g) to salmon inoculated with \textit{L. monocytogenes} and stored at 5 °C delayed, but did not prevent growth of \textit{L. monocytogenes} in vacuum-packs. In order to improve shelflife, brined shrimp are typically produced with the addition of sorbic and benzoic acids. Concerns about the use of these organic acids have led researchers to explore the potential of using bacteriocins for their preservation. The effectiveness of nisin \textsubscript{Z}, carnocin \textsubscript{UI49}, and a preparation of crudebavaricin A on shelflife extension of brined shrimp was evaluatedby Einarsson and Lauzon (1995).

5. Hurdle technology to enhance food safety

The major functional limitations for the application of bacteriocins in foods are their relatively narrow activity spectra and moderate antibacterial effects. Moreover, they are generally not active against Gram-negative bacteria. To overcome these limitations, more and more researchers use the concept of hurdle technology to improve shelf life and enhance food safety. It is well documented that Gram-negative bacteria become sensitive to bacteriocins if the permeability barrier properties of their outer membrane are impaired. For example, chelating agents, such as EDTA, can bind magnesium ions from the lipopolysaccharide layer and disrupt the outer membrane of Gram-negative bacteria, thus allowing nisin to gain access to the cytoplasmic membrane (Abee et al., 1995).

It is well documented that nisin enhances thermal inactivation of bacteria, thus reducing the treatment time and resulting in better food qualities. For example, Bozias et al., (1998) found that addition of nisin (500 to 2500 IU/ml) in media, liquid whole egg, or egg white caused a reduction of required pasteurization time of up to 35%. Budu-Amoako et al., (1999) found that nisin reduced the heat resistance of \textit{L. monocytogenes} in lobster meat and significantly reduced the treatment time compared with thermal treatment alone. The synergistic effect between bacteriocins and other processing technologies on the inactivation of microorganisms has also been frequently reported in the literature. Schlyter et al., (1993) reported synergistic effects between sodium diacetate and pediocin against \textit{L. monocytogenes} in meat slurries. The use of combinations of various bacteriocins has also been shown to enhance antibacterial activity (Hanlin et al., 1993; Mulet-Powell et al., 1998). When used in combination with nisin, leucocin F10 provides greater activity against \textit{L. monocytogenes} (Parente et al., 1998). There has been continued interest in the food industry in using non-thermal processing technologies, such as high hydrostatic pressure (HP) and pulsed electric field (PEF) in food preservation. It is frequently observed that bacteriocins, in combination with these processing techniques, enhance bacterial inactivation. In addition, Gram-negative bacteria that are usually insensitive to LAB bacteriocins, such as \textit{E. coli} O157:H7 and \textit{S. typhimurium}, become sensitive following HP/PEF treatments that induce sublethal injury to bacterial cells (Kalchayanand et al., 1994). Studies in our laboratory also demonstrate that nisin enhances the pressure inactivation of spores of \textit{Bacillus coagulans}, \textit{Bacillus subtilis}, and \textit{C. sporogenes} (Roberts and Hoover 1996; Stewart et al., 2000).
6. Bacteriocins in packaging film

Incorporation of bacteriocins into packaging films to control food spoilage and pathogenic organisms has been an area of active research for the last decade. Antimicrobial packaging film prevents microbial growth on food surface by direct contact of the package with the surface of foods, such as meats and cheese. For this reason, for it to work, the antimicrobial packaging film must contact the surface of the food so that bacteriocins can diffuse to the surface. The gradual release of bacteriocins from a packaging film to the food surface may have an advantage over dipping and spraying foods with bacteriocins. In the latter processes, antimicrobial activity may be lost or reduced due to inactivation of the bacteriocins by food components or dilution below active concentration due to migration into the foods (Appendini and Hotchkiss 2002).

Two methods have been commonly used to prepare packaging films with bacteriocins (Appendini and Hotchkiss 2002). One is to incorporate bacteriocins directly into polymers. Examples include incorporation of nisin into biodegradable protein films (Padgett et al., 1998). Two packaging film-forming methods, heat-press and casting, were used to incorporate nisin into films made from soy protein and corn zein in this study. Both cast and heat-press films formed excellent films and inhibited the growth of L. plantarum. Compared to the heat-press films, the cast films exhibited larger inhibitory zones when the same levels of nisin were incorporated. Incorporation of EDTA into the films increased the inhibitory effect of nisin against E. coli. Siragusa et al., (1999) incorporated nisin into a polyethylene-based plastic film that was used to vacuum-pack beef carcasses. Nisin retained activity against Lactobacillus helveticus and B. thermosphaecta inoculated in carcass surface tissue sections. Coma et al., (2001) incorporated nisin into edible cellulose films made with hydroxypropylmethylcellulose by adding nisin to the film-forming solution. Inhibitory effect could be demonstrated against L. innocua and S. aureus, but film additives such as stearic acid, used to improve the water vapor barrier properties of the film, significantly reduced inhibitory activity. It was noted that desorption from the film and diffusion into the food required further optimization for nisin to function more effectively as a preservative agent in the packaged food. Another method to incorporate bacteriocins into packaging films is to coat or adsorb bacteriocins to polymer surfaces. Examples include nisin/methylcellulose coatings for polyethylene films and nisin coatings for poultry, adsorption of nisin on polyethylene, ethylene vinyl acetate, polypropylene, polyamide, polyester, acrylics, and polyvinyl chloride (Appendini and Hotchkiss 2002). Bower et al., (1995) demonstrated that nisin adsorbed onto silanized silica surfaces inhibited the growth of L. monocytogenes.

The efficacy of bacteriocins coatings on the inhibition of pathogens has also been demonstrated in other studies. For example, coating of pediocin onto cellulose casings and plastic bags has been found to completely inhibit growth of inoculated L. monocytogenes in meats and poultry through 12-wk storage at 4 °C. (Ming et al., 1997). Coating of solutions containing nisin, citric acid, EDTA, and Tween 80 onto polyvinylchloride, linear low density polyethylene, and nylon films reduced the counts of Salmonella typhimurium in fresh broiler drumstick skin by 0.4- to 2.1-log10 cycles after incubation at 4 °C for 24 h (Natrajan and Sheldon 2000). Although shelf life was extended in food products as populations of food spoilage organisms were reduced, the primary thrust was towards control of specific anticipated pathogens in the product.
Suggested Reading


Stewart CM, Dunne CP, Sikes A, Hoover DG. 2000. Sensitivity of spores of *Bacillus subtilis* and *Clostridium sporogenes* PA 3679 to combinations of high hydrostatic pressure and other processing parameters. Innov Food Sci Emerg Technol 1:49-56.


Novel Applications of Lactic Acid Bacteria

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1. Introduction

Lactic acid bacteria are among the powerhouses of the food industry, colonize the surfaces of plants and animals, and contribute to our health and well-being. LAB are gram-positive usually non-motile, non-spore-forming rods and cocci. These are gaining importance due to their “generally recognized as safe” status, long-term use in food and beneficial, probiotic properties. Genera that comprise the LAB are Lactobacillus, Leuconostoc, Pediococcus, Lactococcus, Streptococcus, Enterococcus, Tetragenococcus, Carnobacterium, and Weisella (Table 1). Besides their technological properties in food production, LAB can confer beneficial properties to their hosts, as probiotics. Beyond being probiotic, certain strains of LAB are able to produce specific beneficial compounds in foods such as vitamins, low calorie sweeteners, antioxidants, polysaccharides and minerals.

Table 1 Characteristic of lactic acid bacteria

<table>
<thead>
<tr>
<th>Genus</th>
<th>Lactobacillus</th>
<th>Enterococcus</th>
<th>Lactococcus</th>
<th>Leuconostoc</th>
<th>Pediococcus</th>
<th>Streptococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Rods</td>
<td>cocci</td>
<td>cocci</td>
<td>Cocci</td>
<td>cocci in tetrads</td>
<td>cocci</td>
</tr>
<tr>
<td>CO₂ from glucose*</td>
<td>±</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Growth</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>−</td>
</tr>
<tr>
<td>at 10°C</td>
<td>±</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>at 45°C</td>
<td>±</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>in 6.5% NaCl</td>
<td>±</td>
<td>+</td>
<td>−</td>
<td>±</td>
<td>±</td>
<td>−</td>
</tr>
<tr>
<td>at pH 4.4</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>at pH 9.6</td>
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<td>+</td>
<td>−</td>
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<tr>
<td>Lactic acid configuration</td>
<td>D, L, DL</td>
<td>L</td>
<td>L</td>
<td>D</td>
<td>L, DL</td>
<td>L</td>
</tr>
</tbody>
</table>

http://textbookofbacteriology.net/lactics.html

2. B-Vitamin Production by Lactic Acid Bacteria

The vitamins are disparate group of compound; they have little in common either chemically or in their metabolic functions. Nutritionally, they form cohesive group of biomolecules that are required in the diet in small amounts for maintenance of normal health and metabolic integrity that contributes to healthy lifestyle.

2.1 Riboflavin

Riboflavin (vitamin B2) plays an essential role in cellular metabolism, being the precursor of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) both acting as hydrogen carriers in biological redox reactions.
involving enzymes such as nicotinamide adenine dinucleotide (NADH) dehydrogenase. Deficiency of riboflavin leads to loss of hair, inflammation of the skin, sore throat, hyperaemia edema of oral and mucous membranes, cheilosis, glossitis, cataract development, migrane prophylaxis and decrease in haemoglobin status. Several studies report a link between homocysteine levels and cardiovascular diseases. Riboflavin has been traiditiona Alythesised for food and feed fortification by chemicals means but past decade has witnessed emerging information about commercial competitive biotechnological processes. A study has described the screening of riboflavin-producing strains from different fermented milk products obtained in the Vellore region of India. The selection of spontaneous roseoflavin-resistant mutants was found to be a reliable method to obtain natural riboflavin-overproducing strains of a number of species commonly used in the food industry. The toxic riboflavin analogue roseoflavin was used to isolate natural riboflavin-overproducing variants of the food-grade micro-organisms Lactococcus lactis Lactobacillus plantarum, Leuconostoc mesenteroides and Propionibacterium freudenreichii. Recently, LAB were obtained from durum wheat flour samples and screened for roseoflavin-resistant variants to isolate natural riboflavin-overproducing strains. Two riboflavin-overproducing strains of Lact. plantarum were isolated and used for the preparation of bread (by means of sourdough fermentation) and pasta (using a prefermentation step) to enhance their vitamin B2 content. The applied approaches resulted in a considerable increase in vitamin B2 content (about a two and threefold increase in pasta and bread, respectively), thus representing a convenient and efficient food-grade biotechnological application for the production of vitamin B2-enriched bread and pasta. The bioavailability of the riboflavin produced by this strain was similar to that of pure riboflavin demonstrating the usefulness of this strain for the development of riboflavin-enriched fermented foods. These strain could be further exploited for the enhanced production of riboflavin using various strain improvement strategies to develop a better starter culture for the fermented food industry.

2.2 Folate

Folate, an important B-group vitamin, participates in many metabolic pathways such as DNA and RNA biosynthesis and amino acid inter-conversions. Health of multi-cellular organisms such as humans starts at the individual cell level: if our cells are healthy so are we. Healthy cells, in turn, depend on the continued, faultless replication of our DNA. Folates possess antioxidant properties that protect the genome by inhibiting free radical attack of DNA in addition to their role in DNA repair and replication mechanisms. Folate deficiency has been implicated in a wide variety of disorders from Alzheimer's to coronary heart diseases; osteoporosis, increased risk of breast and colorectal cancer, poor cognitive performance, hearing loss, and of course, neural tube defects. The proper selection and use of folate producing microorganisms is a novel strategy to increase “natural” folate levels in foods. Numerous researchers have reported that LAB, such as the industrial starter bacteria Lactococcus lactis, S. thermophilus, and Leuconostoc species have the ability to synthesize folate whereas many Lactobacilli species happen to consume folate. The ability to produce folate can differ remarkably between different LAB (2 to 214 μg/L folate). It is now known that not only the yogurt starter cultures and L. lactis have the ability to produce folate but also this important property exists in other LAB species as Lb. acidophilus, Leuconostoc lactis, Bifidobacterium longum, and some strains of Propionibacteria, well-known vitamin B12 producer, can also produce large amounts of folate. Majority of folate produced by L. lactis and Leuconostoc spp. being intracellular is not excreted into the milk and hence is lesser bioavailable while S. thermophilus produces folate extracellularly during milk fermentation. Similarly, probiotic microorganisms as
Propionibacteria spp. and Bifidobacteria spp. are also well-known folate producer but majority of folate produced is intracellular and in the case of Propionibacteria it also consumes folate from the medium. Moreover, the major limitations of use of these organisms for biofortification of folate include their requirement of strict anaerobic conditions for folate production and possibilities of folate utilization by co-cultures when used as adjunct starter. *S. thermophilus* known to produce folic acid during growth in milk, which is a functional attribute. *S. thermophilus* has a strain-specific ability of folate production and has been reported to produce higher quantity of folate in comparison to other LAB, majority of which is excreted into milk and has been reported to be the dominant producer, elevating folate levels in skim milk, while lactobacilli have been found to deplete the available folate in the skim milk. Yet fermentations with mixed cultures showed that folate production and utilization by the cultures is additive. Tomar and others (2009) found culture NCDC177 (35 μg/mL) to be the best folate producer among the *S. thermophilus* cultures available at Natl. Collection of Dairy Cultures, Natl. Dairy Research Inst., Karnal (Haryana, India).

2.3 Vitamin B₁₂

The term vitamin B₁₂ is generally used to describe a type of cobalt corrinoid, particularly of the cobalamin (cbl) group. In strict terms, vitamin B₁₂ is the form of the vitamin obtained during industrial production and which does not exist naturally. Animals, plants and fungi are incapable of producing cobalamin; it is the only vitamin that is exclusively produced by micro-organisms, particularly by anaerobes. It was shown that *Lact. reuteri* CRL1098 was able to metabolize glycerol in a B₁₂-free medium; this being the first hint that a LAB might be able to produce cobalamin. The chromatographic analysis of the intracellular bacterial extract of *Lact. reuteri* CRL 1098 confirmed that this strain was able to produce a cobalamin-like compound with an absorption spectrum closely resembling that of standard cobalamin but with a different elution time, while cobalamin production was confirmed using different bioassays. Genetic evidence of cobalamin biosynthesis by *Lact. reuteri* CRL 1098 was then obtained through the use of different molecular biology techniques, and it was shown that at least 30 genes are involved in the de novo synthesis of the vitamin. Recently, a reuterin-producing strain of *Lactobacillus coryniformis* isolated from goat milk was characterized and was shown to produce acobalamin-type compound. Vitamin B₁₂ deficiency and depletion are common in underdeveloped/developing countries, particularly among the elderly and are most prevalent in poorer populations of lower socioeconomic status around the world. Food-cobalamin malabsorption is responsible for about 60%–70% of the cases among elderly patients, and pernicious anemia accounts for 15%–20% of the cases while other causes (nutritional deficiencies, hereditary disorders and malabsorption) collectively claims for <10%. Researchers have found that *H. pylori* gastritis, resulting in food-cobalamin malabsorption, is found as a major cause of vitamin B₁₂ deficiency in a wide range of age groups. Hence B₁₂ supplementation alone can never be an effective approach in the treatment of vitamin B₁₂ deficiency as long as *H. pylori* associated gastritis is intact to the patient. Nowadays, there is a considerable interest in search of alternative/adjunctive therapies against *H. pylori* which can reduce the antibiotic associated outcomes and increase compliance to patient. In this odyssey, probiotics have been found as a “possible tool” for eradication of the infection and an appreciable no. of reports have come out with their positive anti *H. pylori* effect. After taking in to consideration these two points, probiotics can be thought of using selectively against *H. pylori*. Microorganisms most commonly used in clinical practice are lactic acid-producing bacteria (*Lactobacillus spp*) and Non lactic acid bacteria (*Bifidobacterium* and *Bacillus*).
After taking into consideration of both vitamin B₁₂ deficiency and removal of *H. pylori*, one of its potent causes, *Lactobacilli* can be looked upon as a biological companion of pharmaceutical therapies, which are mostly accompanied with unfavorable side effects. *Lactobacilli* can produce vitamin B₁₂ de-novo and show improvement in B₁₂ related hematological and physical and biochemical markers.

3. Low-Calorie Sugars Produced by Lactic Acid Bacteria

Firstly, food-grade microorganisms and their products are directly applicable in food products, without any restriction. Secondly, there is no need for a careful separation of products and microorganisms, which would be the case if microorganisms are not of food grade. Thirdly, some lactic acid bacteria are claimed as beneficial in the gastrointestinal tract. Mannitol production by those bacteria may strengthen their health-promoting ability. Low-calorie sugars have been a recent addition and have attracted a great deal of interest of researchers, manufacturers, and consumers for varied reasons. These sweeteners also getting popularized as low-carb sugars have been granted generally recommended as safe (GRAS) status and include both sugars and sugar alcohols (polyols) which in addition to their technological attributes (sugar replacer, bulking agent, texturiser, humectant, cryoprotectant) have been observed to exert a number of health benefits (lowcalories, lowglycemic index, anticariogenic, osmotic diuretics, obesity control, prebiotic). Some of these sweeteners successfully produced by lactic acid bacteria include mannitol, sorbitol, tagatose, and trehalose and there is a potential to further enhance their production with the help of metabolic engineering. These safe sweeteners can be exploited as vital food ingredients for development of low-calorie foods with added functional values especially for children, diabetic patients, and weight watchers. Mannitol, sorbitol, and erythritol are naturally occurring sugar alcohols. Mannitol is produced by bacteria, yeasts, fungi, algae, and several plants. This polyol might help these organisms to cope with different environmental stresses such as osmotic and oxidative stress. Sorbitol is produced by a variety of both plants and microorganisms. Erythritol production is usually associated with yeasts but has also been reported for some lactic acid bacteria (LAB). All these polyols — mannitol, sorbitol, and erythritol — display properties that are beneficial to human health as they are non - metabolizable, insulin - independent sweeteners, or low - calorie sugars, which make them applicable in dietetic and diabetic food products. In addition, mannitol is used in the pharmaceutical industry as a powerful osmotic diuretic agent and as an osmotic agent for decreasing brain and cellular edema. Mannitol biosynthesis through bacterial fermentation has become an interesting alternative to existing chemical production. Furthermore, the capability of certain LAB, belonging to both homofermentative andheterofermentative species, to synthesize mannitol offers the possibility of in situ production in foods. For this reason, different fermentation technology -based strategies for improving mannitol production by LAB have been reported. To date, 93 – 97 mol%mannitol yields are reached using a bioprocess witha heterofermentative LAB strain.

4. Glutathione

LAB have taken centre stage in perspectives of modern fermented food industry and probiotic based therapeutics. These bacteria encounter various stress conditions during industrial processing or in the gastrointestinal environment. Such conditions are overcome by complex molecular assemblies capable of synthesizing and/or metabolizing molecules that play a specific role in stress adaptation. Thiols are important class of molecules which contribute towards stress management in cell. Glutathione, a low molecular weight thiol antioxidant distributed widely in eukaryotes and Gram negative
organisms, is present sporadically in Gram positive bacteria. However, new insights on its occurrence and role in the latter group are coming to light. Some LAB and closely related Gram positive organisms are proposed to possess glutathione synthesis and/or utilization machinery. Also, supplementation of glutathione in food grade LAB is gaining attention for its role in stress protection and as a nutrient and sulfur source. Owing to the immense benefits of glutathione, its release by probiotic bacteria could also find important applications in health improvement. Different species of LAB have evolved specialized mechanisms to deal with the normally encountered stress conditions in particular niches. These mechanisms essentially involve intricate maneuvering and interplay of various pathways and biomolecules which support the growth of the organism in their respective transient environment. Thiols, distributed widely in biological systems, are one such important class of compounds engaged in stress protection. Important thiol compounds are glutathione, γ-glutamylcysteine, bacillithiol, mycothiol etc. Glutathione, a tripeptide, is ubiquitous in eukaryotic system, found widely in Gram negative bacteria but was known to be scarcely present in Gram positive bacteria.

5. Exopolysaccharides

The role of microbes in producing fermented dairy products has evolved from a chance discovery to a highly elaborated process involving the production of specialized “starter” of bacteria that function consistently in large cultures. The primary function of almost all starter cultures is to develop acid in the product. The secondary effects of acid production include coagulation, expulsion of moisture, texture formation and initiation of flavor production. Starters also help in imparting pleasant acid taste, conferring protection against potential pathogens and providing a longer shelf life to the product. The food industry uses polysaccharides as thickeners, emulsifiers, gelling agents and stabilizers. The demand for these ingredients is mostly met by alginates, carrageenan, cellulose, pectins, starches etc. There is a growing interest for all-natural, healthy food products. Moreover, in various countries the amount of stabilizers being used is regulated. In this respect the lactic acid bacteria (LAB) have great potential, as many of its representatives are known to produce exopolysaccharides (EPS). EPS from LAB are an alternative class of biothickeners, having potential for development and exploitation as functional food ingredients with both health and economic benefits. Consumer demand for products with low fat or sugar content and low levels of additives, as well as cost factors, make EPS a promising and viable alternative as these contribute to texture, mouth-feel, taste perception and stability of the final product. A large variety of EPS can be produced by LAB employed for production of fermented dairy products. In particular for the production of yoghurt, drinking yoghurt, cheese, fermented cream, milk-based desserts, EPS producing LAB play a significant role. They play a major role in the production of fermented dairy products in Northern Europe, Eastern Europe and Asia. EPS producing lactic cultures have also been successfully used for the manufacture of Nordic ropy milks. Scandinavian fermented milk drinks display a firm thick, slimy consistency and these rely on the souring capacity of mesophilic ropy strains of Lactococcus lactis subsp. lactis and ssp. cremoris and concomitant production of heterotype EPS for texture.

6. Selenium

Selenium is a trace element which is essential for normal functioning of both humans and animals. It happens to be the only mineral that qualifies for a Food and Drug Administration (FDA)-approved qualified health claim for general cancer reduction incidence. The Se residue is essential for catalytic activity as it takes part in catalysis. Thus, Se enhances immunity, growth, reproductive performance, and inhibition of
pathogens. Se deficiency has been associated with the decreased activity of glutathione peroxidase. Food is the main source of Se for the human population. Se levels in foodstuffs such as cereals, grains, fruits and vegetables are relatively low and cannot meet people’s daily dietary requirement. Presence of selenium in food is generally reflected by its levels in soil. It has been reported that Se bound in organo-metallic complex are much better absorbed by the body than if they are taken in the inorganic form. In a recent publication, biotransformation of Se (IV) has been studied, when the process of lactic fermentation was carried out with bacteria *Lactobacillus* in the presence of increasing amounts of Se (IV) to produce Se-enriched yogurt. The main species found were selenocystine (SeCys2) and methylselenocysteine (MeSeCys). It was found that various *Lactobacillus* species could concentrate Se intracellular as seleno-cysteine in biomass and suggested that Se-enriched lactobacilli could provide a means of concentrating selenoproteins and can be used as an organic selenium source for dietary supplementation.

A number of studies have been conducted and resulted in the production of selenium enriched biomasses. Lactic Acid Bacteria have been recognized to have the ability to synthesize biomolecules containing Se. It has been reported that Seleno-*Lactobacillus* could be used as an organic selenium source. In a US patent application another genera, *Pediococcuspentosaceus* has been reported to produce both organic and inorganic fractions of selenium and feeding of such selenium enriched bacteria to animals showed higher levels of glutathione peroxidase activity in tissues indicating an increased absorption of and retention of selenium. A US patent have been rewarded for the technology of food preparation, food supplement and nutraceutical product comprising selenium enriched biomass of viable Lactobacilli isolated from faecal samples. Advantageously, the microorganism used in the process of the technology may be selected from the group consisting of the following species: *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacteriumbifidum*, *Streptococcus thermophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus* and *Bifidobacteriumlongum*. The selenium source is the sodium hydrogen selenite (NaHSeO3) as well, but in powder form. For the inoculation one or the mixture of the following strains: *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Lactobacillus casei* were added. After mixing all components in medium, cans or buckets were placed into the shaking incubator for 36-48 hours at 37°C. In the end of the fermentation process, dense, selenium rich pink coloured yoghurt was prepared.

7. **Galactooligosaccharides**

Beta-galactosidases are glycoside hydrolases and are categorized within the glycoside hydrolase families (GH) 1, 2, 35 and 42. GH2 b-galactosidases catalyze the hydrolysis of lactose into glucose and galactose, and the transfer of the galactosyl-moiety to suitable acceptors. In the presence of high concentrations of lactose, GH2 b-galactosidases produce galactooligosaccharides (GOSs) (Hsu, GOSs are commercially employed in infant formula and have shown therapeutic properties reducing the adherence of enteropathogenic *E. coli*. One of the main differences between probiotics and prebiotics is that probiotics are viable food components whereas prebiotics are nonviable food component. Use of probiotics is a way to replenish bacteria levels in the gut with external microorganisms. Food products having probiotic components may contain bacteria that are not necessarily indigenous to the human gut so once in the gut they have to compete to find a place among established, colonized bacteria. Probiotics can also be destroyed by the contents of the gastrointestinal tract. So probiotics cannot be used in wide range of food products because of their viability issue. On the other hand, prebiotics are non digestible, remain intact through the digestive system and act as food for already
established microflora. They are added to dairy products, table spreads, baked goods, and breads, breakfast cereals and bars, salad dressings, meat products, and some confectionery items. So prebiotics overcome many of the traditional limitations of introducing probiotic bacteria into the GI tract. Therefore, using prebiotics is arguably a more practical and efficient way to manipulate the gut microflora. The beta-galactosidases (beta-Gals) of Lact. reuteri L103 and L461 proved to be suitable biocatalysts for the production of prebiotic galacto-oligosaccharides (GOS) from lactose. Another study employed b-galactosidases present in disrupted crude cell extracts (CCEs) and whole cells of LAB and bifidobacteria for formation of galactooligosaccharides (GOSs) and heterooligosaccharides (HeOSs) from lactose and the acceptor carbohydrates mannose, fucose, N-acetylglucosamine (GlcNAc) and sialic acid. CCEs and whole cells successfully produced up to three HeOSs with mannose, fucose or GlcNAc in addition to GOS, but did not utilize sialic acid as acceptor. Heterologously expressed b-galactosidases of S. thermophilus and Lactobacillus plantarum hydrolysed the novel HeOSs and confirmed, for the first time, fucose as an acceptor carbohydrate. LAB CCEs and bifidobacteria CCEs and whole cells are suitable sources of b-galactosidases that can be used to synthesize novel HeOSs with potentially expanded functionality in addition to GOSs.

8. Conclusions

LAB have been extensively used for centuries as starter cultures to carry out food fermentations and are looked upon as burgeoning “cell factories” for production of host of functional biomolecules and food ingredients. As such abilities are recognised to be strain specific, it can be an attractive strategy to bioprospect prolific metabolite producing strains from their diversified natural niche and enhance their ability by microbiological and biotechnological interventions. Such strains can be used for microbial synthesis of biomolecules as an effective and attractive strategy for their delivery as a functional bio-ingredient through foods to meet the daily recommended intake of human population. This information opens the way to increase metabolites concentrations in fermented foods through judicious selection of the microbial species and cultivation conditions. The food industry has taken the step to use this information for selecting various health promoting metabolites producing strains as part of their starter cultures to produce fermented products with elevated levels of these essential compounds. Such products would provide economic benefits to food manufacturers as increased ‘natural’ metabolite concentrations would be an important value-added effect without increasing production costs. Consumers would obviously benefit from such products as they could increase their functional food ingredients intake while consuming these as part of their normal diet.

9. Suggested Reading


Personnel Hygiene and Occupational Standards for Food Safety System

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*Model Dairy Plant, #Dairy Microbiology Division

1. Introduction

The contaminants enter the food chain via environment, water, food contact surfaces, raw materials, or through personnel handling food. Anyone responsible for running a food business must ensure that adequate provision and procedures are put into place to ensure that all employees are aware of the potential food safety risks that may arise. Through increasing the knowledge and skills that individuals have in regards to potential food safety hazards, risk can be greatly reduced and public health protected.

The Occupational Standards for food safety personnel identify and create uniform food safety practices and procedures which will enable employers and workers to meet regulatory, 3rd party auditor customer and consumer needs; consistently and repeatedly. These standards lay a general foundation on which food processors can tangibly measure their workers and their workers’ current knowledge and skill against a national standard. This enables companies to adequately hire, train and up-skill the workers so they can continue to effectively perform in the workplace. This article highlights the codes of hygiene for food safety personnel and how the development and adoption of occupational standards can ultimately provide safer food within the complex world of the food safety system.

2. Food handlers: a potential source of contamination

The food handler may transmit the disease causing organisms directly to food via atomized particles extruded from the nose/mouth while talking, sneezing or coughing over raw material, packing material, food itself or indirectly through doorknobs, pencils, books, washroom fittings, clothing, money, knives and equipment. Parts of the body that contribute to the contamination of food include the skin, hands, hair, eyes, mouth, nose, naso-pharynx, respiratory tract, and excretory organs. Pathogenic bacteria associated with food handlers include Streptococci Staphylococci Intestinal microorganisms, Salmonella, Shigella, Escherichia coli, cholera, infectious hepatitis and infectious intestinal amoebas. These micro-organisms are of public health concern because they can contribute to serious health illnesses.

3. Codes of personnel hygiene

3.1 Food handlers responsibilities

3.1.1 Health

Employees should maintain a good physical, mental and emotional health to reduce respiratory or gastrointestinal disorders and other physical ailments.

3.1.2 Illnesses/wounds

Injuries including cuts, burns, boils and skin eruptions should be reported to employer abnormal conditions such as respiratory system complications like head cold, sinus infection, bronchial or lung disorders. Intestinal disorders such as diarrhea should also be
reported to the employer so that work adjustments can be made to protect food from the handler’s illness or disease.

3.1.3 Hygiene

Personal cleanliness that should be practiced includes daily bathing, hair washing at least twice a week, wearing clean undergarments and uniform and maintenance of short & clean fingernails.

3.1.4 Hygiene Practices

- To prevent biological hazardshabis such as scratching the head or other body parts, tobacco chewing, eating smoking in food processing areas should be stopped
- The mouth and nose should be covered during coughing and sneezing
- Hands should be kept out of food. Food should not tested from the hand nor should it be consumed in food production areas
- The hand should be washed thoroughly with soap under warm running potable water effectively (20 sec. friction wash) after visiting the toilet, using a handkerchief, smoking, handling soiled articles and handling money (see fig below)
- Rules such as ‘no smoking”, ‘no pan/tobacco chewing’ should be followed and other precautions related to potential contamination should be taken
- Where required employees must use disinfectant hand dips.-When gloves are used employees should wash their hands before putting on gloves. Multi use gloves should be washed & sanitized while single use should be discarded and replaced after the employee touches any non-product compact surface. Gloves worn outside the food production area should be discarded before returning to the food production area.
- Employee should use footpaths containing sanitizer when entering food production areas
- Personnel effects and street clothing must not be kept in food handling areas & must be stored in a manner to prevent contamination of food.

![Fig-1 Proper hand washing techniques](image-url)
Hygiene Practices: To prevent physical hazards: The following actions are not allowed in food areas: Wearing jewellery, Holding toothpicks, matchsticks or other objects in the mouth, Placing pencils, pens behind the ears, Wearing false eye lashes or fingernails, Carrying objects above the waistline (eg. thermometers, pens, flashlights, I cards, Non permanent badges)

3.2 Responsibilities of Supervisors

3.2.1 Supervision
Supervisors should observe employees daily for infected cuts, boils, respiratory complications & other evidences of infections.

3.2.2 Self-Hygiene
Supervisors & managers should set an example by their own high level of hygiene & good health.

3.2.3 Hygiene Monitoring
Targets for hygiene are of little value unless they are monitored. The cleanliness can be assessed by objective examination of hand. This is carried out in microbiological laboratory by taking washings from the hands by means of sterile cotton-wool pads and inoculating the washings on to nutrient media. For this the surfaces of palms of both hands and the fingers are rubbed at least five times with each pad and then used to rub the spaces between the fingers, the nails and the space underneath the nails.

The results of the examination should be communicated to the workers.

![Swab](image1.png)
Swab

![Swabbing of hands](image2.png)
Swabbing of hands

![Plating on selective media](image3.png)
Plating on selective media

**Fig-2** Hygiene monitoring of Personnel

3.3 Management Responsibilities

3.3.1 Facilities and infrastructure
Safe food handling requires appropriate infrastructure and personnel accessories.

- Cloakrooms: Separate cloakrooms are required for top clothing, ordinary outer-wear, and special working clothing.
Showers: For best results of personnel hygiene the showers bath should be arranged in such way so that the worker cannot reach at place of work without passing under the shower.

Welfare Facilities: Lunchrooms/ change rooms / Toilets: These facilities must be separate with self-closing doors and should not lead directly into food processing areas.

Manicure room: In food plants arrangements for ensuring good personal hygiene among the workers a manicure room should be available where a manicurist looks after the nails of the staff.

Hand washing: Processing areas must contain a sufficient number of conveniently located hand-washing stations with properly trapped waste pipes connected to drains. In processing areas, hand washing stations should have knee /foot/ elbow operated faucets and remote operated liquid soap dispensers. Sanitary towel services or Suitable drying services must be made available.

Garments: Gloves, footwear’s, smocks, should be made of impermeable material, in good repair and easily disposable. Pockets above the waist may be removed or sewn shut. Only zippers / Velcro may be used as the fasteners on shirts, coats.

3.3.2 Adoption of occupational standards: Need of the hour

3.3.2.1 Food business operators are to ensure

- That food handlers are supervised and instructed and/or trained in food hygiene matters commensurate with their work activity;
- That those within the business responsible for developing and maintaining food safety management procedures, e.g. HACCP based procedures, are appropriately trained.

National Occupational Standards (NOS) can be used for defining the range of skills and knowledge coupled with varying job roles. NOS are statements of ‘competency’ developed to reflect the relevant skills and knowledge associated with specific job tasks.

The following tables outline the NOS developed for the food and drink manufacturing industry and identify the job roles for which they will be relevant

<table>
<thead>
<tr>
<th>Level 1 – Operative roles</th>
<th>Knowledge based component</th>
<th>Job relevance</th>
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</table>
| Maintain personnel hygiene standards in food manufacture | Awareness of food safety in food and drink manufacturing:  
- Keep him/herself clean and hygienic  
- Keep the work area clean and hygienic  
- Keep the product safe | Relevant to food handlers at all levels. May also be applicable to regular contractor and agency staff |
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<th>Level 2 – Operative roles</th>
<th>Knowledge based component</th>
<th>Job relevance</th>
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| Maintain personnel hygiene standards in food manufacture | Principles of workplace food safety in food and drink manufacturing:  
- Take personal responsibility for food safety  
- Keep him/herself clean and hygienic  
- Keep the work area clean and hygienic  
- Keep the product safe | All operative roles who deal directly with food stuffs. May also be applicable to longer term agency staff |

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<tr>
<th>Level 3 – Supervisory and technical roles</th>
<th>Knowledge based component</th>
<th>Job relevance</th>
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</table>
| Raise food safety awareness in manufacture | Principles and practices of food safety management in food and drink manufacturing:  
- Ensure compliance with legislation  
- Apply and monitor good food safety practices  
- Implement food safety management procedures  
- Contribute to staff training  
- Food safety auditing  
- Safe procurement and supply in food and drink manufacturing:  
- Implement and maintain procurement procedures  
- Schedule supply  
- Implement and maintain product traceability  
- Implement and maintain an audit trail  
- Receive goods | Supervisory staff, team leaders, managers |
| Monitor food safety at critical control points in manufacture | | Supervisory staff, team leaders, managers |
| Contribute improvement of food safety in manufacture | | Supervisory staff, team leaders, managers |
| Control and monitor safe supply of raw materials and ingredients in food manufacture principles | | Supervisory staff, team leaders, managers |

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<tr>
<th>Level 4 – Managerial roles</th>
<th>Knowledge based component</th>
<th>Job relevance</th>
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| Allocate roles and responsibilities for food safety management in manufacture | Key requirements of food safety management procedures  
Roles and responsibilities required for implementation of procedures  
Key principles for reducing the risks associated with relevant food safety hazards | Management and roles concerned with food safety management procedures and their implementation |
| Implement controls to manage safe food sources and products in manufacture | Records applicable to food | |
| Assess operation for | | |

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<th>Effectiveness and compliance with food safety standards in manufacture</th>
<th>Safety management systems</th>
<th>Roles concerned with food safety management procedures and their implementation</th>
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<td>Report on compliance with food safety requirements in manufacture</td>
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<td>Analyse food safety hazards and risks in manufacture</td>
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<td>Produce action plan to control food safety hazards and risks in manufacture</td>
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<tr>
<td>Develop policy and guidelines to manage food safety in manufacture</td>
<td>Importance of effective food safety management</td>
<td>Management and roles concerned with food safety management procedures and their implementation</td>
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<tr>
<td>Develop working practices and procedures to manage food safety in manufacture</td>
<td>HACCP principles and practice and their application</td>
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<tr>
<td>Develop recording procedures to monitor food safety in manufacture</td>
<td>Nature and type of hazards that may occur</td>
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<td>Risk assessment and management techniques</td>
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<td>Methods of hazard control</td>
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<td>Provide guidance on food safety information</td>
<td>Importance of multidisciplinary approaches to preparation of guidelines and systems</td>
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<td>Implement systems for food safety information</td>
<td>Importance of systematic approaches to food safety management</td>
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<td>Role of hygiene procedures</td>
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<td>Best practice within relevant industry sectors</td>
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<td>Key principles of hazard analysis and risk assessment</td>
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<td>Provision of food safety training to staff</td>
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<td>Importance of continuous improvement</td>
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Source: www.improveltd.co.uk

### 3.3.3.2 National Occupational Standard for the HACCP / Food Safety Coordinator Position

Due to the increased prominence and importance of Quality Assurance positions (in which HACCP positions fall under) in the food and beverage processing industry, as well as the growing need for National Occupational Standards, initiatives have been
taken to create NOS for the position of a HACCP Coordinator. The use of such a standard would ensure consistency across and will enable employers to attract and retain qualified personnel; all of which would result in increased productivity and sustainability for the sector. The standard created will be a benchmark for workers and employers, as well as a benchmark upon which the food & beverage processing industry and educational institutions can build. The national occupational standard will have a variety of practical applications, in a streamlined format.

4. Conclusions

The occupational standards highlight the practical competencies that should be developed and the associated knowledge relevant to each activity. They can also be used to help with the design of training/instruction programmes by clearly identifying the key subject areas that need to be covered and by highlighting the appropriate level. The standards are based on industry good practice and will therefore still require one to add information specific to one’s organization i.e. company specific food safety management procedures etc.

The challenge of preparing quality safe food begins with well trained and knowledgeable food industry workers. To truly meet this challenge, National occupational standards could be developed and adopted with a common goal of consistently ensuring safe food for the consumers across the globe.
1. Introduction

Intellectual Property means the legal rights which result from intellectual activity in the industrial, scientific, literary and artistic fields. There are eight different types of intellectual property rights (IPR) which are defined as legal rights granted to a person for creations of mind or intellect having commercial value. These include (i) Patents, (ii) Copyrights, (iii) Trademark, (iv) Industrial designs (v) Layout designs of integrated circuits, (vi) Geographical indications, (vii) Registration of plant varieties and (viii) Trade secrets. These are like any other property but no physical objects.

Patent is an exclusive right given by Government to an inventor for for a limited period of time, for protecting against illegal use of invention. Any invention which can be patented must satisfy three basic criteria: novelty, inventiveness and commercial value.

1.1 Novelty

Any invention for which there is no prior art is called novel invention. The prior art is existing known things on the date of filing patent application. The prior art can be checked from published literature (Journals, books, periodicals, magazines, manuals, thesis, annual reports etc.). Patents filed before the date of filing patent application are under within the definition of prior art. Traditional knowledge is also covered under prior art. Even presentation in conferences can be treated as prior art. A thorough search is essential for deciding whether invention is novel or not. Patent search can be made from following free sites

(i)  [www.uspto.gov](http://www.uspto.gov) (USA)
(ii)  [http://www.patents1ic.gc.ca](http://www.patents1ic.gc.ca)
(iii)  [http://ep.espacenet.com](http://ep.espacenet.com) (Europe)
(iv)  [www.ipaustralia.gov.au](http://www.ipaustralia.gov.au) (Australia)
(v)  [http://www.kipris.or.kr](http://www.kipris.or.kr) (Korea)
(vi)  [www.pfc.org.in/db](http://www.pfc.org.in/db) (India)

Paid sites are-

(i)  [www.patent.office.nic.in](http://www.patent.office.nic.in)
(ii)  [www.wipo.org](http://www.wipo.org)
(iii)  [http://ipindia.nic.in](http://ipindia.nic.in)
(iv)  [www.bpmlegal.com](http://www.bpmlegal.com)
1.2 Inventiveness (Non-obviousness)

The invention should be non-obvious. It means that invention should be obvious to a literate person. A literate person is a person having reasonable knowledge in the subject of invention.

1.3 Commercial value (Utility)

The invention must be of commercial value. It means invention has industrial application.

All the three criteria are essentially for grant of patent and therefore, inventor must carefully apply all these criteria and satisfied before submitting patents application. If any of these criteria is not fulfilled, patent cannot be granted.

2. Grant of patents

Patents are granted by Government and therefore the rights are valid within geographical boundary of country. Prior art has no geographical boundary and therefore, prior art has to be searched globally. A patent granted in India is not protected in other countries. Similarly, patent granted in USA is not protected in India. IPR in case of Patents are protected in countries where patents are granted. The duration of patent in 20 years and is counted from the date of filing patent application. The date is also referred as Priority Date. Patent applications can be submitted when invention is partially or completely worked out. Accordingly, patent applications are divided into two categories namely (i) Provisional Application and (ii) complete specification application.

Provisional application is filed when some work related to invention is not completed. A patent application with provisional specification is valid for 12 months and patent application with complete specification must be submitted within 12 months of filing provisional application. If the same is not done the patent application is treated as withdrawn.

It means that based on filing of provisional application, patent cannot be granted. For the grant of patent, patent application with complete specification must be submitted. The priority date is the date on which provisional or complete application is filed. It means that provisional application helps in obtaining an early priority date.

2.1 Patent of Addition

If an inventor makes an improvement in invention specified in original patent application; ‘patent of addition’ can be submitted. This can be filed after submission of complete specification and is granted after grant of main patent. Patent of addition can be filed even after grant of main patent but priority date is same as that of main patent.

2.2 Divisional Patent

If a patent application contains more than one invention; the applicant may file a separate application for the second invention. The patent application with second invention is divided out of parent application is called Divisional Patent. In Divisional Patent application, a reference to main patent is made.

2.3 PCT international application and PCT national phase application

PCT is an international filing system for patents in which the applicant gets an international filing date in all the designated countries, conferring the late entry (up to 31 months) to the national offices without affecting the priority date. This is a simple and economical procedure for the applicants seeking protection for their inventions in many countries. Indian Patent Office is a Receiving Office for international applications by
nationals or residents of India. An international application shall be filed with the appropriate office of the country or with international bureau. An international application made according to the Patent Cooperation Treaty designating India can enter national phase within 31 months from the priority date of international application or date of filing of international application whichever is earlier. Such an application filed before the Controller in the Indian Patent Office claiming the priority and international filing date is called PCT national phase application. The filing date of the application shall be the international filing date accorded under the Patent Cooperation Treaty.

2.4 Publication and Examination

Application is published after 18 months of submission of patent application in journal of patent office. The patent application is not examined by examiners of patents (in patent office) unless it is published. After publication, request for examination along with fee (Rs. 2500/- for individual and Rs 10,000/- for corporate) is submitted. Examiners may sent examination report to applicant and report has to be appropriately responded back within 12 months. Patent application can be requested for early publication by paying fee (Rs 2500/- for individual and Rs 10,000/- for corporate) and early publication can occur within 3-4 months. After early publication, examination fee is paid and examination starts. This allows saving of 12-15 months.

2.5 Claims

Claims are to be written carefully. If it is not covered in claim part, IPR is not protected. It requires the knowledge of technical matter and knowledge of law and therefore, claims can be better written by lawyer in close consultation with inventor.

2.6 Maintenance of patent

A patent is maintained by paying maintenance fee every year. If maintenance fee is not paid, the patent will cease to remain force and invention becomes open to public. Anyone can use patent if not maintained.
Market Research Techniques: Communicating Consumers’ Voice to Product Development Team

Smita Sirohi
Dairy Economics, Statistics & Management Division

1. Introduction

In the modern day market-led economies where ‘consumer is the king’, an assessment of the customer’s expectations, preferences and aversion becomes a pre-requisite for success of a new product in the market. A new product can have various connotations, such as,

- Innovative and new to the world
- Product lines that allow a company to enter an established market for the first time (the product is new to the company not the market)
- Products that supplement a company’s established products lines (package sizes, flavors, and so on)
- Products that provide improved performance or greater perceived value and replace existing product (improvements in features and benefits of a product)
- Products that provide similar performance at lower cost to the company
- Products that are targeted to new markets or market segments (to be called a new product there must be some changes in the existing product to suit the new segments targeted).

Business entities are continuously on the lookout for new products as existing products are vulnerable to changing consumer needs and tastes, new technologies, shortened product life cycles, and increased domestic and foreign competition.

Market research provides the key inputs required by the technical development group for the setting of appropriate design specifications for the new product or service. This lecture note briefly outlines the various techniques that are used for market research.

2. Market Research: Concept and Objectives

Market research is a systematic process that collects, analyzes and draws conclusions from data gathered from consumers, business owners, or other groups of interest. The goal of market research is to identify and assess how changing elements of the marketing mix impacts customer behavior. As it is based on analysis of qualitative and quantitative data about issues relating to marketing products and services, the market research minimizes risk, provides business intelligence to make informed decisions and therefore, improves the chances of success in launch of a new product or service, fine tuning existing products and services, expanding into new markets, developing an advertising campaign, setting prices, and/or selecting a business location. The term is commonly interchanged with marketing research; however, expert practitioners may wish to draw a distinction, in that marketing research is concerned specifically about marketing processes, while market research is concerned specifically with markets.

3. Coverage of Market Research

Market research examines all aspects of a business environment. It covers both the secondary and primary aspects that are useful for the business planning. The secondary market research includes information about the growth trends in the concerned business sector, size of the target market, presence of competitors, market structure, government
regulations, economic trends, technological advances, and numerous other factors that make up the business environment. The primary research includes key information about customers and prospects, including their demographic profile, the types of features or special services they want, what they like and dislike about specific product or service, how they use the product or service, how often they buy and how much they will pay for the product, etc. The nature of market information that can be useful for the technical product development group is outlined in Box 1.

### Box 1. Market Information requirement for New Product Development

A. General product description
   1. Concept
   2. Anticipated consumer need and type of use for product
   3. Closest competition on market today
   4. Anticipated points of difference from existing competition

B. Marketing position of product

C. Targeted selling price for given unit size

D. Method of preservation and distribution—refrigerated, frozen, shelf-stable

E. Anticipated packaging

F. Anticipated shelf life requirements

4. Market Research Methods

There are many ways to perform market research, of which the commonly used five basic methods are:

4.1 Surveys

The sample that represents the product target market can be surveyed using structured concise and straightforward questionnaires. There are various ways in which sample survey can be conducted.

4.1.1 In-person surveys

They are one-on-one interviews typically conducted in high-traffic locations such as shopping malls. They allow the product developers to present people with samples of products, packaging, or advertising and gather immediate feedback. In-person surveys can generate response rates of more than 90 percent.

4.1.2 Telephone surveys

These are another way of reaching out to the voice of the customer. However, due to consumer resistance to relentless telemarketing, convincing people to participate in phone surveys has grown increasingly difficult. Telephone surveys generally yield response rates of 50 to 60 percent.

4.1.3 Mail surveys

Mail surveys are a relatively inexpensive way to reach a broad audience. They're much cheaper than in-person and phone surveys, but they only generate response rates of 3 percent to 15 percent. Despite the low return, mail surveys remain a cost-effective choice for small businesses.
4.1.4 *Online surveys*

These are simple, inexpensive ways to collect anecdotal evidence and gather customer opinions and preferences. But it usually generates unpredictable response rates and unreliable data, because there is no control over the pool of respondents.

4.2 *Focus groups*

In focus groups, a moderator uses a scripted series of questions or topics to lead a discussion among a group of people. These sessions take place at neutral locations, usually at facilities with videotaping equipment and an observation room with one-way mirrors. A focus group usually lasts one to two hours, and it takes at least three groups to get balanced results.

4.3 *Personal interview*

Like focus groups, personal interviews include unstructured, open-ended questions. They usually last for about an hour and are typically recorded.

4.4 *Observation*

Individual responses to surveys and focus groups are sometimes at odds with people's actual behavior. Observing consumers’ actions in stores, at work, or at home, can throw light on their consumption behavior. This gives a more accurate picture of customers' usage habits and shopping patterns.

4.5. *Field trials*

Placing a new product in selected stores to test customer response under real-life selling conditions can help to make product modifications, adjust prices, or improve packaging.

5. **Voice of Customer**

Voice of the Customer is a market research technique that produces a detailed set of customer wants and needs, organized into a hierarchical structure, and then prioritized in terms of relative importance and satisfaction with current alternatives. Voice of the Customer studies typically consist of both qualitative and quantitative research.

Qualitative research looks at the content, not the numbers. It is unstructured, exploratory in nature, based on small samples, and may utilize popular qualitative techniques such as focus groups (group interviews), word association (asking respondents to indicate their first responses to stimulus words), and depth interviews (one-on-one interviews which probe the respondents' thoughts in detail). Other exploratory research techniques, such as pilot surveys with small samples of respondents, may also be undertaken. Qualitative research methods are designed to talk to a relatively few people in the target audience of interest to primarily plumb the depths and range of buyer attitudes and beliefs. The qualitative research is useful to generate ideas and concepts and to uncover consumer language. It yields valuable insights into customer attitudes and is an excellent way to uncover issues related to new products or service development.

Quantitative market research methods attempt to gauge quantity, for instance when market research answers the question, how many people have certain habits, it entails quantitative market research while the requirement to know why and how of customers behavior is qualitative research. Quantitative research is used to test a specific hypothesis, based on the data collected from large number of respondents using random sampling techniques. As a social research method, quantitative market research typically involves the construction of questionnaires and scales. People who respond (respondents) are
asked to complete the survey. The data acquired for quantitative marketing research can
be analysed by almost any of the range of techniques of statistical analysis, which can be
broadly divided into descriptive statistics and statistical inference. Techniques include
choice modelling, maximum difference preference scaling, and covariance analysis.
Marketers use the information so obtained to understand the needs of individuals in the
marketplace, and to create strategies and marketing plans.

6. **Market Research Studies for Dairy Products: Examples**

This report analyzes the worldwide markets for Dairy Products in US$ Million by the
following product segments: Milk Powder, Butter, Cheese, Ice Cream and Related
Products, Yogurt and Related Products, and Cream. The report provides separate
comprehensive analytics for the US, Canada, Japan, Europe, Asia-Pacific, Middle East,
and Latin America. Annual estimates and forecasts are provided for the period 2007
through 2015. A seven-year historic analysis is also provided for these markets.

The study estimates the latent demand and potential industry earnings for functional dairy
products in over 100 countries around the world incluing India.

6.3 Nestle: Performance, Capabilities, Goals and Strategies in the Worldwide Dairy
Market (2011)
A strategic assessment of Nestle, one of the world’s leading dairy companies, provides
significant competitor information, analysis, and insight critical to the development and
implementation of effective marketing and R&D programs. The report presents a
comprehensive analysis of the company’s performance, capabilities, goals and strategies
in the global dairy market.

6.4 Milk & Dairy Products in India (2009)
This report by Hindustan Studies & Services Ltd. and Infolitics covers all aspects of
production, consumption and exports of milk and dairy products. Starting from an
analysis of the economic and social environment, the report looks at drivers and
impediments of growth of dairy sector in India. It gives statistics and insights into
domestic market dynamics of liquid milk, dairy fats (butter and ghee), curd, processed
cheese, table butter as well as for traditional Indian dairy products like khoa, paneer,
chhana etc. The report has detailed country-wise export statistics for each dairy product
has forecast of production, consumption and exports for years up to 2012 / 2013.

China Dairy Market Monthly Report covers authoritative and comprehensive info and
data. China Dairy Market Monthly Market is prepared based on the data information from
Ministry of Agriculture, State Statistics Bureau, State Customs Bureau and local finance
publications, also up to date info from local dairy associations, dairy product associations,
daughter processing enterprises, packaging enterprises, which forms a net work from
government, dairy farms, dairy processing enterprises and dairy traders to local markets.

6.6 Dairy Products - Top 5 Asian Markets (2011)
This package contains top 5 Asia dairy product analyses from the following countries:
China, Japan, India, South Korea, and Indonesia. These market research reports offer an
in-depth perspective on the actual market situation, trends and future outlook for dairy
products in top 5 Asian markets.
1. **Introduction**

The product development is a process, which is under the company’s control and the modifications in its features and capabilities and values can be inbuilt during the development phase. But, the purchase of any product is determined by many other variables and is beyond the company’s control. The product purchase may be affected by many variables including the income, price, the market, the competitors etc. The companies have to make sure that the product will be accepted by the final customers. One of the ways for a company to make sure that the product will be accepted by the target customers is to undertake a test marketing programme before the formal launch of the product.

2. **The new product development process**

The process of new product development involves various steps. The objective of these steps is to avoid product failures by continuously reviewing the prospects for the new product/product idea, and give the company several discrete points in time at which the decision to drop the product and cut their losses. The most expensive mistake on the part of a company is to launch a product which ultimately proves unsuccessful. Figure 1 depicts the new product development process.
3. Ideation (Idea generation)

Idea generation is the creative process of generating, developing and communicating new ideas and it is also known as “fuzzy front end”. The objective of idea generation is to gather as many ideas as possible. The possible sources may include internal as well as external sources:

<table>
<thead>
<tr>
<th>Internal sources</th>
<th>External sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>• The research and development department of the company and other organizations</td>
<td>• The market intermediaries</td>
</tr>
<tr>
<td>• The top management of the company</td>
<td>• Customers</td>
</tr>
<tr>
<td>• Sales and marketing staff</td>
<td>• Competitors</td>
</tr>
<tr>
<td>• Company employees</td>
<td>• Marketing consultants</td>
</tr>
<tr>
<td>• Public and media</td>
<td></td>
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</tbody>
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4. Screening of ideas and concept testing

At this stage, the ideas are to be screened in terms of their relative strengths and weaknesses. The screening must exclude those ideas which are weak in terms of their chances of market success or their potential returns on investment. The product ideas must be evaluated against the company's mission and objectives. The product development policy of the company must cover the dimensions related to compatibility, market potential and financial objectives. The objective of idea screening is to eliminate unsound/less potential concepts prior to allocating resources to them. Idea screening must cover

- The benefits to customers in the target market
- Size and growth potential of the target market?
- Competitors’ response to the product launch
- The technical feasibility to manufacture the product?
- Product profitability

The concept testing is to estimate customer reactions to a product idea before committing substantial funds to it, it could be a response to the product idea (a concept test), its proposed uses, to the physical properties and characteristics of the product itself (a product test).

5. Analyzing business prospects

The business analysis involves an assessment of the total capital investment cost, the likely return-on-investment and the payback period. The objective at this stage is develop the guesstimates for the expected sales volumes, revenues and expenses. The analysis involves the use of a set of pro forma financial statements showing what future income and expenses would be generated if a new product idea was fully developed and marketed.

6. Technical development of the product

In this stage, the product concept is translated into a prototype or trial formulation in the case of a food product, animal health treatment or agrochemical. Generally, one product idea is likely to undergo technical development. The technical staff will use the
prototype/trial formulation to assess the feasibility of producing the product and arrive at the most cost effective method of manufacturing.

7. Market testing
Market test will provide a real-world exposure for product evaluation and its marketing programme. The objective here is to determine whether or not the potential profit opportunity outweighs the potential risks by a considerable margin. Although more often the test marketing is associated with consumer markets, it can also be applied to industrial markets.

8. Final product launch
After establishing the success of all the previous stages, the company decides about the final product launch. The launch must be effective and must lead to consumer awareness about the product attributes. It is true that the study of the new product development process is not only about innovation, it also helps in understanding the adoption and diffusion processes. The innovators tend to be risk-takers. The inherent characteristics of an innovation process greatly influence its adoption and rate of its adoption.

9. Test marketing
Test Marketing can be defined as the product development stage where the product and its marketing plan are exposed to a carefully selected sample of the population for deciding whether the product will be accepted by the customer or not. It is an experiment conducted in a test market before the final launch and comprises of real-life buying situations, without the buyers knowing they are participating in an evaluation exercise. In test marketing, the product is sold to the ultimate market to understand the consumer reaction and may last from few weeks to several months depending on the product. The test marketing generally involves higher costs and therefore may not be suitable for all products.

9.1 Considerations in test marketing
- The composition of the population and the selected sample in the test market must be demographically similar to the target market in which the product will be sold. The important considerations here include the population size, demographic composition of the population, lifestyle considerations and the competitors’ situation.
- The test market should be small enough to make advertising effective. At the same time, it should be large enough to have proper representative of the main market.
- The test market should be distant enough from the main markets of the product so that any negative effect of the product during test marketing may not impact the main market.

9.2 The Uses of Test Marketing
The test Marketing is done with the purpose of collecting data during test marketing so that it can be extrapolated for its use over the entire region for a launch. These decisions will help the company understand the problems/modifications in the product which need to be addressed before the final launch. The following aspects can be evaluated with the help of test marketing:
- In understanding the effectiveness of the advertising
- The likely problems in various distribution channels of the product
9.3 Requisites for Test Marketing

The company must be clear on its objectives and what it aims to achieve from the test marketing. Following are the requisites for test marketing:

- In which market, the test marketing will be undertaken?
- Which population characteristics will be studied and tested
- How long the test marketing will be conducted?

The test marketing reduces risk in marketing. The final launch of a product means that the company has to manage a large budget for its launch. For the new products the channel members and the sales force needs to be trained and the marketing activities need to be performed along with the launch of the product.

10. Marketing strategies for new products

Marketing is considered to be the key to the success of any business. A new firm needs to develop a written marketing plan with timelines to identify the market and potential demand for the novel food products. Marketing strategies can be summed up in the 4 P’s: product, price, place and promotion. The following discussion will consider each of the 4 P’s of marketing.

11. Product related strategies

Before launching the new food products, the firms must ensure the regulatory environment for the product. There are number of dimensions attached to the product:

- What is the composition of the novel product?
- What does the consumer wants from the product and what features does it have to meet those needs?
- Where and how the customer will use the product?
- What form the product will take after processing?
- Will the product be cooked, dried, frozen, solid or liquid?
- What kind of recipes are involved?
- How the product is differentiated over other products already existing in the market?

Once the product processes and forms have been determined, the packaging and labeling must be designed. Proper packaging and labeling of the product are required to ensure shelf life and are also important marketing tools. Now, packaging is more than just something to hold the product until a customer buys it. The package’s appearance, shape, style and convenience are the reasons responsible for consumer purchases. When many competing products are available in the market, the package becomes the silent salesman on a store shelf. The packaging needs to catch the attention of the consumer. The package and label can become the brand image for the product and the company. This is particularly very important in case of new food products because the consumer seeks greater information in a new and differentiated product. The ultimate goal is to create a brand in the mind of the consumer that will promote loyalty and encourage repeat purchases.
12. Pricing strategies
The second “P” of marketing mix is price. There are certain questions which are important in pricing

• What is the value of the product to the consumer?
• Whether the consumer is price sensitive and what is the price elasticity of demand?
• What discounts are to be offered to trade customers?
• How will the price compared to the competitors?

Price depends on many factors, the important ones are the cost of producing and delivering the product to the market. The price must cover the total cost, return a profit, and be competitive in the market place.

Cost accounting plays an important role in setting the selling price. Good recordkeeping is the key to estimate the total cost involved in product production. Maintaining a good recordkeeping system will provide the required information for accurately pricing a product for market. The price should consider the cost first, and then adjustments can be made for other considerations. Some other considerations that can affect price setting are the competitors’ price, seasonality of product, volume of purchases, specialization, and location.

13. Strategies related to physical distribution
The third “P” in marketing is place--that is, getting your product in the consumer’s basket. Place involves distribution from the point of manufacture to the final consumer. The key questions in this are:

• Where do the consumers look for the product?
• How can the right distribution channels be assessed?
• What are the distribution channels adopted by the competitors and how those can be more effective?

Different levels or stages of distribution create opportunities for different markets and strategies for marketing the product. The marketing may be done through the marketing middlemen like wholesalers, distributors, dealers, retailers etc. Depending upon the type of the product, sometimes even institutions like restaurants, hospitals, schools and other large-scale food providers may be targeted. This requires a different marketing plan than selling directly to a retail store or directly to final consumers. Selecting a particular marketing option will require different packaging and labeling considerations which in turn will affect the margin between processing costs and selling price.. Food service organizations usually require products in larger size units because they are involved in large-scale food production in order to manage operations. Each step in the distribution and supply chain offers services for marketing the product. The place to market a product can be any one of the different stages of distribution or a combination of several market channels. A marketing plan should help identify the market place or combination that offers the most potential profitability.

14. Promotion strategies
The fourth “P” of the marketing is promotion. Certain key questions associated with promotion are:
• Where and when the marketing messages are to be provided to the target market?
• How to position the product?
• Which is the best promotional mix?
• What promotional mix is adopted by the competitors and how does that influence the choice of your promotional mix?

These are just a few of the questions that can help in building a promotion campaign. Advertising is one way to promote a product and can be accomplished through a multitude of media outlets. Various media outlets used for advertising are radio, television, newsprint, magazines, signage, and the Internet. However, advertising can be expensive and even cost prohibitive for small companies in some situations. Building relationship with local media people and encouraging them to write information stories about the business or product can also be very helpful. The marketer should keep the media informed of any special activities associated with the product or business. Participating in various fairs like India International Trade Fair, Dilli Haat, Kissan Mela, Dairy Mela etc. is a great way to get consumers to try the product, and retailers generally like any activity that encourages customer traffic. Promotion is all about the product exposure and brand awareness. Marketers need to be creative and be visible on repeated occasions in order to make the customer aware of the product.

15. Selected Reading


Cost Cutting Measures in New Product Development

Latha Sabikhi
Dairy Technology Division

1. Introduction

The increasing inflation in the recent years have forced food manufacturers all over the world to keep prices as low as possible. While profitability is a key issue for sustainability of any enterprise, low costs are imperative in consumer satisfaction and retention. There is an increasing demand for more competitive production by small and medium food producing companies, which have limited facilities to compete with the multinational producers. Besides the value-added products the other main issue that governs their sustainability is finding a solution to reduce their manufacturing costs.

2. Cost reduction at the manufacturing site

The five broad priority areas identified to achieve a reduction in manufacturing costs are:

- raw materials and packaging costs
- energy costs
- water consumption
- effective utilization of manpower and
- Maintenance.

The three major approaches to achieve these are:

- operations analysis
- innovative ideas and
- waste prevention.

Food manufacturers have paid considerable attention to operations analysis for improved manufacturing efficiency and waste prevention. A variety of specially developed operational analytical tools such as Six Sigma, Lean Manufacture and Cost-Benefit Analysis are applied in modern enterprises. There are specialists these days who provide expert advice on these issues. Such approaches have delivered savings as high as 20% of manufacturing cost in well-executed programmes.

Coming out with innovative ideas for cost reduction is a demanding task for the R&D department of any food manufacturing enterprise. As the routine activities of product, process and package development are usually hectic, innovative cost reduction does not always receive the full attention it deserves. To reach full savings potential, the R&D manager has to keep abreast with developments in cost reducing solutions offered by the various suppliers in the marketplace and formulate a systematic procedure to evaluate and select them, finally applying the selected options to the complete product line. On several occasions, this may also involve challenging the existing processes on specific issues to get hidden cost saving leads. As this is done best by an external unbiased participant, companies often resort to using external specialists for specific parts of their cost saving projects. The investment is rapidly recovered by the extra savings achieved.

A holistic R&D approach to cost savings in product, process and packaging would take into account, design, optimisation, rationalisation, efficiency, harmonisation, flexibility and quality improvements. Product quality defects, such as off-taste, oxidation, colour stability, storage stability, shelf-life extension and manufacturing variability are issues that need particular attention.
Reduction in formulation and packaging costs are two main aspects of covered under innovative ideas, as they lead directly into increased product profitability. However, such changes are often risky, as they may result in product changes which are negatively perceived by the consumer. Although specific expertise in product development quality assurance and consumer surveys is essential, not all companies have the necessary knowledge and resources. Cost reduction in food product development should allow a firm to reduce the cost of the product, but still provide the same benefits that the old, existing product has provided to the customer. It is important to keep in mind that any changes in the product or package brought about by cost reduction should not diminish the brand equity or the product quality perception by the user. Some ways to reduce manufacturing costs without introducing any product changes perceivable by the user include the application of ingredient analogues, product re-design, purchasing optimisation, process rationalization and co-packing. These measures need not be communicated to the consumer. However, in certain situations where, besides reducing manufacturing costs, the cost reduction would result in products with additional new benefits, it would require communication to the consumer. For instance, health benefits of lower fat content or environmental benefits with less packaging needs to be communicated to the consumer with effective marketing tools in order to sustain footholds in the marketplace.

Food manufacturing companies should conduct dedicated cost reduction programmes for the existing product range at certain time intervals, in order to maximize profitability. There are certain modern tools used by managers for this purpose, including Formulation Value Analysis and Big Scale Value Analysis. Harmonisation of raw materials or product reformulation for improved health is also another way to achieve lower costs. For example, in order to target the obese customer, recent introduction of low fat products have resulted in reducing costs to certain degree.

Waste prevention is an ongoing process in all companies. Improved waste monitoring could save food processing plants lakhs of rupees annually. This activity is usually the responsibility of the plant or works manager. Wastes may be in terms of raw materials wasted or spoiled due to faulty handling and storage practices, ordering excess material due to inability to forecast the amount of material needed, packaging losses, wastage due to returns and also wastage of service accessories (water, electricity etc.) in the manufacturing facility. The presence of food components in food processing waste streams has long been recognised as a problem for downstream treatment plants and receiving waters. Economically, these food solids and chemicals are considered product losses that have been paid for once, and then paid for again as they are carried from a plant along with the waste water. A plant's waste load can be decreased substantially by controlling the amount of water used and reducing the amount of product lost into the sewer. Stopping pollution at its source is less expensive and more practical than a waste treatment programme at the end of processing operations.

The job satisfaction and emotional status of the employees contribute tremendously to the efficiency of manufacturing. As higher efficiency leads to lower costs, it is important that managers keep their workforce professionally satisfied. Increased involvement in the decision making process, accountability, profit sharing and periodic training programmes to achieve improvement in efficiency are some measures to improve manpower utilization.

Regular preventive maintenance of equipment is important in keeping efficiency high. Obsolete equipment and their parts should be auctioned off periodically. All equipment
should run at full capacity. It is not wise to install large capacity plants and equipment to carry out small scale processes. All product, steam and water leakages should be plugged immediately. Adequate safety measures should be installed to ward off expenses incurred as a result of hazards.

3. Cost reduction by the ingredient suppliers

While producers have thought of new methods to reduce manufacturing costs, suppliers have also responded, with new functional blends that bring additional benefits to replace expensive materials. Suppliers of ingredients continuously endeavour to come up with better and cheaper products to offer to their customers and to the marketplace. The food ingredients area showcases new high-tech branded blends and ingredients with improved functionalities that address multiple benefits. Savings in product formulation reportedly range between 10 and 20%, depending on the product. Some examples of such ingredients are:

- blends that enhance volume as well as stability of whipped products
- fat replacing blends that also deliver improved mouthfeel and storage stability to the product
- flexible ingredients that can be used in multiple product lines by the same manufacturer, aiming to reduce costs and inventory and to simplify purchasing and storing processes
- novel analogues that contribute to increased stability to replace scarce or expensive ingredients, and
- process accelerating ingredients that are cheaper.

4. Cost reduction by suppliers of packaging material

Techniques such as combining structural design and new and lighter materials offer the same functionality and appearance while significantly reducing packaging material use. Approximately 20% reduction is reported in plastic for soft drink bottles by adopting these means. The three-fold advantage derived is savings in terms of material costs, and transport costs as well as in environmental penalties and taxes. Digital printing techniques have reduced set-up and printing costs tremendously. Sleev ing, rather than printing or in-mould labelling allows much better package appearance at a lower cost. Package development linked to process and equipment design offers better efficiency. Careful combination of package shape, size and palletisation also offers savings in production.

5. Conclusion

Cost reduction in a food manufacturing plant can be achieved by efficient utilization of resources, quality assurance of products, proper maintenance of equipment, reducing water and energy wastage and having a happy and satisfied workforce. Cost reduction at many times is achieved at the cost of food safety, quality and environment. Enterprises and consumer should both accept the higher price of food safety. An efficient system both at the manufacturers and the consumer level would look at reduced manufacturing costs, better quality, increased consumer satisfaction, increased profitability as well as sustained existence in the marketplace.

6. Selected Reading

The Art of Scientific Writing and Communication

Meena Malik
Associate Professor (English)

1. Introduction
Communicating science is as important to the scientific process as designing, conducting and analyzing the experiment itself. A scientific experiment, irrespective of its spectacular results, is not completed until the results are published. In fact, the foundation of science is based on the premise that original research must be published. This is the only way by which new scientific knowledge can be authenticated and then added to the existing data base that we call science. Scientific writing is used in all fields of science, technology, agriculture, engineering and social sciences. Any branch of knowledge requiring a systematic study involves the use of scientific and technical writing for the purpose of recording and reporting information. Scientific writing is different from creative writing as it deals with scientific facts and does not present an imaginary view of reality. Scientific reporting and writing is objective in content and systematic in form. It is always precise, exact, and to the point so that it may have the desired effect on the reader and lead to the required action. In the field of education and research, journals publish technical material on specialized fields and are circulated amongst the scientists and scholars. All these writings must conform to the rules of scientific and technical reporting so that they are properly understood and appreciated. All types of articles such as Technical Articles; Semi-technical Articles; Popular Articles; Research Papers; Dissertations and Theses, and Technical Bulletins are covered under the ambit of Scientific/Technical Writing.

2. Format of Scientific Writing and Communication
The nature of the subject, the purpose of the scientific report and the reader for whom the report is written determine the form and structure of the communication. Every written communication has a specific purpose and a specific audience. It should be carefully planned and constructed to fit both. Every scientific communication has one certain clear purpose: to convey information and ideas accurately and efficiently. The objective requires that the communication be: (1) as clear as possible; (2) as brief as possible; and (3) as easy to understood as possible. Scientific writing, if it is to be effective and efficient, must be designed for the needs and the understanding of a specific reader or group of readers. One must have adequate knowledge of the educational and professional background of the readers. The language and style of the writing depends, to a great extent, on the academic and professional background of its readers. We need to have an idea of what the reader expects from the report and his level of understanding. Writing should be aimed at the average reader, but should also cater to those at either extreme of the range. It should interest the more knowledgeable reader and be intelligible to the reader who is less familiar with the subject.

3. Organization of Scientific Reporting and Writing
There is no precise formula for the organization of scientific reports. The material in any report should be presented in an order that leads logically towards a conclusion or
conclusions. The various sections of the report are organized so that each of them has its logical conclusions. Almost every scientific communication should have three functional elements. This does not mean that it should be divided by boundaries into three distinct parts. But functionally it should have a beginning, middle and an end. The beginning orients the reader and supplies him with background material, so that he will see how the subject of the paper fits into the general scheme of things. It prepares the reader for the main presentation of information-the middle. The beginning is often called Introduction, which states the purpose of the investigation and describes the basic scheme of the procedure or methods used. It orients the reader by supplying as much historical background as necessary and then describing the present problem. The middle is usually the longest part of the report. The end is sometimes labeled conclusions. It brings together the various subjects that have been discussed and shows their relationships with each other and with broader fields. It leaves the reader with some thoughts about one phase of it. This end section makes the exposition come to a logical and an obvious termination.

4. Research Paper as a Form of Scientific Writing

A scientific paper is a written and published report describing original research results. A research/scientific paper is primarily an exercise in organization. Each scientific paper should have, in proper order, its Introduction, Materials and Methods, Results and Discussion (IMRAD). Early journals published descriptive papers. IMRAD pattern slowly progressed and came to be adopted by most of the journals in the latter half of the nineteenth century.

IMRAD pattern is an effective way to proceed to answer these four questions:

- Introduction: What question was studied?
- Materials and Methods: How was the problem studied?
- Results: What are the findings?
- Discussion: What do these findings mean?

5. Essential Parts of the Scientific Paper

Good organization is the key to good writing. A scientific paper is highly stylized with following distinctive and clearly evident components parts.

5.1 Title

The title should be short but sufficiently explanatory for the reader to know what the report is about. It should be appropriate, brief, indicative and informative. The title is likely to be reprinted in bibliographies and subject indexes, stored in bibliographic data bases. It has to be ensured that the title does not promise more than what is in the article. At first glance, on the basis of the title a paper/article may be rejected or opened up.

A good title is the one that:

- Contains a few words/ is short
- Describes the contents of the paper accurately/ is clear
- Describes the subject as specifically as possible
- Avoid abbreviations, formula and jargon
- Is easy to understand
- Contains key words for the benefit of information retrieval system.
5.2 By-Line
The by-Line normally has two elements; the names (s) of the author (s) – that is, the person (or persons) who contributed materially to the research being reported; and the name (s) of the institution (s) where the research was done. If the author has moved to another institution, his current address may be given in the footnote.

5.3 Abstract
An abstract is a very important portion of an article and is best placed before the text of the manuscript so that the reader might comprehend the essence of the author’s research. Abstract is generally restricted to 250 words or even less. The abstract should be informative, give a succinct condensation of the article and complete in itself and be intelligible without reference to the text, figures or tables. It should include principal objectives and scope of investigation, methodology/techniques employed and the contribution of the particular piece of work towards new knowledge. The abstract is placed on a separate page or immediately after the title. It should be typed in single space and centered on the page leaving equal space on the left and right side.

It should:
- Avoid use of abbreviations.
- Be written in the past tense as it refers to work done.
- It should never give any information or conclusion that is not stated in the paper.
- It must be accurate with respect to figures quoted in the main text.
- It should not cite any references (except in rare cases)

5.4 Introduction
Introduction should be relatively short. This should give background information, define the nature and extent of the problem, relate research to previous research, explain the objectives and highlight the need for present investigation. It should reveal the background, important and specific objective of research work undertaken. It should also point out lacunae in earlier research in that field.

- Detailed review of literature should be avoided. Cite peer-reviewed scientific literature or scholarly reviews. Avoid general reference works such as textbooks.
- Three to four citations may suffice to corroborate a statement.
- Keep the use of abbreviations to a minimum in this part.
- Use present tense but the work reviewed of others may be presented in past tense.
- Define any specialized terms or abbreviations

5.5 Materials and Methods
This section describes subjects, materials and methods used, including experimental design, in sufficient detail to enable other scientists to evaluate the work or to duplicate research procedure. The usual sequence for experimental studies is design of the experiment, subjects (plant, animal, human), materials, procedures and methods for observations and interpretation. Unnecessary details are avoided. If well known methods are used without modification, simply methods are named, or at the most papers describing them are cited. Methods are described in sufficient details in case modifications of previously described methods are used. Methodology be explained in such a manner that the entire experience could be repeated by other research workers by adopting the technology method mentioned in this section. The planning and presentation of the body of the report should be done with
the reader in mind. He should be able to learn what you have done and how and what you have found and where your findings stand in relation to existing knowledge. And, above all, no reasonable question that may arise in the mind of the reader should be left unanswered.

5.6 Results

This section provides facts and figures duly supported by statistical analysis. The results are given in orderly and coherent sequence including pertinent mean data and their standard errors. Results should be related with other standard and recent work on the subject, drawing conclusions and logical implications. All the tables, graphs, histograms and photographs etc, should be arranged in a logical order.

5.7 Discussion

The purpose of the discussion is to clarify matters developing from the work described earlier in the report. The contents of this section should be directly based on the work contained in the body of the report. No new information should be introduced in the discussion.

This part in fact is an attempt to show how far the objectives set out or the assumptions made at the beginning of the research work/ experiments have been achieved. It is interpretation of the data. Data should be referred to and discussed but should not be repeated under discussion. In the discussion, all possible explanations should not be hypothetical but based on the work of other scientists. If the observations/ results of the other workers are to be contradicted, ensure that the experimental conditions, materials and methods of your experiment were the same as used in experiment that to be contradicted. This section is meant to explain, justify or defend anything new or unusual about the results and make sure that the approach is logical and arguments are objective. In any report or article, the discussion should be brief and to the point.

5.8 Conclusions

The main findings and inferences flowing directly from your work are listed in this section. This section is brief and does not present new arguments. All conclusions should be unconditional and unambiguous, and listed in order of importance; with the most important one first.

5.9 Recommendations/Proposals

Recommendation is distinct from the conclusion in that the conclusion is a direct outcome of your work whereas a recommendation need not be so. A recommendation is to some degree a matter of opinion and should not be included under conclusions. Recommendations should be made with complete objectivity and be based as soundly as possible on demonstrated facts. The understanding, integrity and seriousness of the author are judged through these recommendations. Proposals too are suggestions based on the findings of the work, but made with less conviction than are recommendations. A proposal needs further study before it is accepted and acted upon.

5.10 Acknowledgments

Some journals include an acknowledgements section in which credit is given to persons for grants in aid and to those who have helped with research or in writing the article. In short reports, acknowledgements may be placed at the end of the report and in large reports, at the beginning. Sometimes acknowledgements are made in the preface to the report.
5.11 Literature Cited or References

Rarely is it possible to write a report without consulting the work of others, published or otherwise. Reference to the work consulted will, on the one hand, enable the reader to refer to the work if necessary. If the number of references is large or if the same work is referred to frequently, a separate list of references will be necessary but if the number of references is small they may be given in footnote. The listing may be in alphabetical order or in the sequence in which the reference occurs in the article. Where the number of references is large, the alphabetical order is desirable.

6. Some Important Language Points and Skills

The primary objective of scientific writing is to transmit information briefly, clearly and efficiently. This can be achieved only through simple, direct and unadorned style. Following are some of the some of the language skills that make scientific writing effective:

- Use simple language.
- Use a short word rather than a long word, a plain and familiar word rather than a fancy or unusual word.
- Use a concrete word rather than an abstract word.
- Avoid complex sentence structure.
- Always keep in mind that the paragraph is the essential unit of thought.
- Avoid Verbosity and long-winded phrases.
- Use specialized terms only in specific fields, but not for the wider group of the people outside that field.

7. Authorship

Authors should include persons who can defend the intellectual content, including data and conclusions. The author is responsible for generating at least part of the intellectual content (conception or design, data analysis and interpretation) or for drafting, reviewing or revising critically the intellectual content. Authors must comply with the following rules when submitting the manuscript for publication:

- The manuscript is not under consideration elsewhere and the research will not be submitted elsewhere until a final decision has been made by the journal.
- The manuscript is a trustful, original work without fabrication, fraud or plagiarism.
- The authors have made an important scientific contribution and are familiar with the primary data.
- The authors have read the manuscript and take responsibility for its content, and understand that if the paper, or part of it, is found to be faulty or fraudulent, they share responsibility.
- All funding sources supporting the work and all institutional or corporate affiliations of the authors must be acknowledged.
• The authors must certify that they have no commercial association that might pose a conflict of interest in connection with the submitted paper.

7.1 Order of Authorship

Some journals use the alphabetical order for authorship. Most of them assume an order based on each author’s importance to the study:

• The first author is primarily responsible for collecting and analyzing data, and writing.
• The last one, an established investigator, assumes the overall responsibility for the study.
• The middle authors are listed according to their order of importance to the study.

8. Conclusions

Scientific writing is objective in content and systematic in form. It has to be clear, simple and well ordered communication to transmit the scientific facts. Scientific writing and reporting has a specific purpose and a specific audience. It should be carefully planned and prepared keeping the reader in mind. It is the art of making the subject intelligible to others, which requires invaluable mental discipline and in turn enhances clear thinking.
1. Introduction

Functional Foods are the fastest growing segment in world food market of value added products. However, “Functional Foods” and “nutraceuticals” currently lack on uniform definition. Functional foods are believed to include products that provide essential nutrients often beyond quantities necessary for normal maintenance, growth and development, and/or other biologically active components that impart health benefits or desirable physiological effects. With its strong tradition for healthy eating, India ranks among the top ten buyers of functional foods.

Nutraceuticals are becoming the most essential components of rapid growing health movement across the globe. Significance of nutraceuticals assume altogether different dimension in our country where rapid rise in malnutrition and incidences of non-communicable diseases is posing newer challenges. It is costing not only 2-3% to National GDP, but adversely affecting the quality human resource as well. In India 20% of the total population and 44% of children (below 5 years of age) are undernourished and these figures are even worst than the poorer nations of Sub-Sahara region. Attempts have been made over the years to address the problem of malnutrition, but still huge challenge is ahead of us. Inspite of introduction of iodized salt long back, about 10% people are still suffering with goitre, which is twice the cut-off level of WHO. Widespread Iron deficiency has affected the 79% children and 56% women and every 1/5th maternal death is attributed to anaemia. Another side of the story is related to imbalanced nutrition that has enhanced the burden of diabetes and cardiovascular diseases (CVDs) with estimation of 30 and 32 million patients respectively. India has a meager share (only 1%) of global nutraceutical market which is worth Rs. 5148 billion. However, the growth rate of Indian nutraceutical market is much higher (i.e. 18%) as compared to global market, which is only 7% (Sharma and Nair, 2012). Among the functional foods dairy based products occupy an important place, probably because of the well perceived health benefits associated with consumption of milk and milk nutrients. The present article is an attempt to discuss the present status of functional dairy foods, the issues and challenges in their designing and commercialization.

2. Milk nutrients for general well being

With changing life-style, there has been increase in the number of chronic diseases at alarming rate. Despite the top most producer of milk globally, the per capita availability of milk is quite variable across the length and breadth of nation. The consumption pattern of milk and milk product also vary from region to region and the highly imbalance might also have contributed significantly towards the malnutrition. India has attained the first rank in numbers of persons suffering or prone to diabetes, cardiovascular diseases (CVDs) and cancer. Moreover, incidences of infectious diseases are also on rise. One of the common reasons for these diseases could be attributed to impaired or weak immune system. Role of milk nutrients specially the minor milk proteins such as β–Lactoglobulin, α-Lactalbumin and lactoferrin, in modulating the immune system is well documented. However, how different processing interventions affect the nutritional and therapeutic
virtues of milk nutrients is a matter of thorough investigations. Thermal treatment not only effective in improving the digestibility of milk proteins, but heating of milk is also known to produce various intermediates as Maillard reaction products. Many of these maillard reaction products have been identified with anti-oxidant potential; on the other hand these also have been implicated in allergic responses and carcinogenesis. Therefore, research investigations pertaining to processing induced changes on nutritional and therapeutic potential of various categories of processed dairy products should be initiated. Better availability of added nutrients in milk and milk nutrients have offered newer opportunities for the fortification of bioactive such as essential fatty acids, micronutrients and therapeutic amino-acids. Recent findings related to anti-obesity and anti-carcinogenic role of conjugated linoleic acids (CLA) in animal models have suggested the enrichment of CLA content in milk and milk products. Enhancement in CLA level through dietary manipulation or processing mediated interventions would appears promising. Milk mining through advanced technological interventions (separation technologies) has enabled us to isolate the wide array of components present in milk and so far more than 500 compounds have been identified so far. Recent developments in clinical sciences also contributed significantly in elucidating the mechanisms associated with therapeutic virtues of these molecules.

3. Functional dairy foods for infants and children: Critical for preventing malnutrition

Breast milk or mother’s milk is probably the first and most diverse kind of functional food which a new born consumes. It is designed by nature to provide all essential nutrients and therapeutic components in desired amount and also in best bio-available form. The bioactive components present in colostrum and mature milk include nutrients, minerals, trace elements and pre-vitamins as well non-nutrients (mostly bioactive) such as immunoglobulin, hormones, growth factors (Insulin-like growth factors), cytokines, prostaglandins, enzymes, lactoferrin, transferrin, nucleotides, polyamines and human milk oligosaccharides (HMO) (Blum and Baumrucker, 2008).

Breastfeeding continues to offer health benefits into and after toddlerhood. These benefits include; lowered risk of Sudden Infant Death Syndrome (SIDS), faster mental development, lowered incidences of cold & flu, lowered risk of asthma and eczema, decreased risk of obesity later in life, and decreased risk of developing psychological disorder. Breast milk provides a wide variety of proteins that have unique compositional and physico-chemical characteristics that is highly suitable for neonates. In addition to these, they also exhibit several extra-nutritional roles to promote the development and well being of infants.

The exact integrated properties of breast milk are not entirely understood and everyday new scientific evidence is emerging that make the task of infant food formulators more tedious. However, mimicking the composition and functionality of mother’s milk is quite a daunting task and efforts have so far done are concentrated towards balancing the nutritional content of cow or buffalo milk for infant feeding. Emerging trends in infant formula are: incorporation of long chain poly-unsaturated fatty acids (LUFA), fortification with prebiotics, trace elements (iron, zinc), addition of milk /soy protein hydrolysates and nucleotides (Alles et al., 2004; Thompkinson and Kharb, 2007). But the type of bioactive molecules to be added in infant formula and their concentration still need extensive investigations. Furthermore, a strong need is felt to develop infant formula, for pre-term and neonates suffering with specific metabolic disorders.
During the last decade, manufacturers of infant food have made impressive advancement in developing technologies for up-gradation of nutritional characteristics of infant formulas similar to human milk. However, susceptibility of bottle fed infants to enteropathogenic micro-organisms still responsible for the largest health risk. The protective effect of human milk for infant, inspite lack of transfer of antibodies from gut into the circulatory system indicates to direct protective role of human milk in the ecological system of the intestinal tract. During early infancy, risk for mortality and morbidity from common pathogen like coliform, salmonella and shigella are very high.

4. Milk nutrients as precursor for bioactive components

Casein and lactose two major milk nutrients which may serve as starting material for the production of nutraceuticals such as bioactive peptides, lactulose, lactitol and galactooligosaccharides. A great amount of work has been dedicated to these health promoting components. Biologically active peptides are of particular interest for food and pharma industry because they have been shown to play different physiological roles, including opioid like activity, antimicrobial, immunomodulatory and antihypertensive. These peptides could be generated during hydrolysis by digestive or microbial enzymes. Microbial enzymes from lactic acid bacteria (LAB) have demonstrated to be able to liberate theses peptides from milk proteins, in various fermented milk products (Korhonen and Pihlanto, 2007). Upon oral administration bioactive peptides may affect the major body systems- namely the cardiovascular, digestive, immune and nervous systems. The potential of certain peptides sequences to reduce the risk of chronic diseases or boost natural immune protection has aroused a lot of scientific interest over the past few years. These beneficial health effects may be attributed to known peptide sequences exhibiting, e.g., antimicrobial, antioxidative, antithrombotic, antihypertensive and immunomodulatory activities (Sasaki and Kume, 2007). Certain commercial peptides and other functional dairy foods have been listed in Table 1.

Lactulose is an isomer of lactose, which is formed during heating of milk in small amounts. Lactulose plays a role in proliferation of Bifidobacterium spp. that has a positive relationship with human health. Investigation regarding the effect of incorporation of lactulose in infant formula on the intestinal bifidobacterial flora in rats indicated that 0.5 – 1.0% lactulose content in formula had no adverse effect on the absorption and retention of nitrogen, calcium, phosphorus and iron from the formula by bifidobacteria flora. Health benefit associated with lactulose among elderly is its ability to act as mild purgative, thus it helps in reducing the growth of ammonia producing organism. This particular property of lactulose has successfully utilized by medical practitioners in the treatment of portal systemic encephalopathy and chronic constipation. Human milk contains various types of oligosaccharides and most predominant among them is galactooligosaccharides (GOS). The presence of GOS is breast milk is linked with higher bifidobacterial count in infants (Sangwan et al., 2011). GOS are produced during the lactose hydrolysis via glycosyl transferase mediated activity of certain microbial strains. These galactooligosaccharides were earlier considered as unwanted products but now they are considered as prebiotics because they function as bifidobacteria growth promoting factors, reduce risk of colon cancer, prevent bone loss and lower serum cholesterol concentration.

5. Designing of novel dairy foods with non-dairy bioactive and ingredients

Fusion trend has also influenced the dairy food formulations and blending of raw materials form different food groups wither for better nutritional status or for the improvement of quality of resultant product has gained momentum in last few decades.
Development of low calorie and/or no fat products required substantial alteration in formulations and removal of milk fat and sugars or salt have numerous undesirable consequences on quality attributes of finished products. Search for fat, sugar and salt replacers have resulted in availability of various alternatives, which could be effective in minimizing or completely eliminating these macromolecules. Artificial sweeteners including aspartame, acesulfame-k, sucralose, saccharin etc., have also been permitted by the regulatory agencies in wide range of dairy products. Studies conducted at NDRI revealed that it is possible to incorporate these intense sweeteners in combination with bulking agents and fat replacers in traditional dairy products without posing any safety threat. Inulin, Fructooligosaccharides (FOS), Simplesse (modified whey protein), Oatrim (oat based fat replacer) and certain modified starches are fast becoming the essential ingredients in functional dairy products such as yoghurt, yoghurt drinks, ice creams, cheeses, spreads etc. Availability of safety and toxicity data related to these ingredients also enhance consumer faith in products based on these ingredients. Inulin and other non-digestible polysaccharides also have well documented health benefits, acting as prebiotic by assisting the proliferation of bifidobacteria and lactobacilli and improving the overall gastrointestinal health (Roberfroid et al, 1993). Other claimed benefits include increased calcium absorption with positive effects for bone health, a lowering of serum lipids with relevance for heart health, a positive effect on feeling of satiety with potential positive consequences for weight management, a potential effect to enhance resistance to infections and to stimulate the immune system. Phytochemicals, novel plant metabolites could be an ideal substrate for the manufacture of functional dairy foods. Among more than 1000 phytochemicals few such as carotenoids, flavonoids, phytosterols, phytoestrogens, glucosinolates and soluble fibres have been utilized in certain dairy products. These phytochemicals primarily act as antioxidants and perform putative functions mainly in lifestyle-associated mortality and morbidity including CVD, diabetes and cancer.

Phytosterols exhibit anti-inflammatory, anti-neoplastic, anti-pyretic and immune-modulating activity. In the body, phytosterols can compete with cholesterol in the intestine for uptake, and aid in the elimination of cholesterol from the body. Saturated phytosterols appear to be more effective than unsaturated ones in decreasing cholesterol concentrations in the body. These actions reduce serum or plasma total cholesterol and low-density lipoprotein (LDL) cholesterol. In mammals, concentrations of plasma phytosterol are low because of their poor absorption from the intestine and their faster excretion from liver, and metabolism to bile acids, compared to cholesterol. Phytosterols have been successfully incorporated in yoghurt, cheese, dairy spreads and milk beverages.

5. Probiotic dairy foods

The major focus in development of milk based therapeutic products has been towards the incorporation of probiotic microorganisms that harbour our gastro-intestinal (GI) tract and are frequently associated with health promoting attributes. Probiotic foods contain viable probiotic microorganisms in requisite number in suitable matrix and their viability & metabolic activity should be maintained through processing, packaging, storage till it is consumed. The global probiotic products market generated $15.9 billion in 2008. More than 500 probiotic F&B products have been introduced in the past decade. These products have received varying levels of success, mostly in congruence with their overall health benefits. A number of scientific publications are emerging on selection, incorporation of probiotic cultures in dairy products and impact of unit operations on their viability during processing. The survivability of probiotics in complex GI tract and demonstrated health benefits in consumers is of great concern among researchers and processors (Mercenier et al, 2010).
Several factors have been reported to influence the viability of probiotics in dairy foods and their subsequent implantation in host intestine. Certain processing and formulation interventions have been found to be effective in enhancing the viability of probiotics. Through In-vitro and In-vivo trials the possible mechanisms for therapeutic aspects of probiotics have been revealed. These mechanisms are mainly related to anti-microbial activity, anti-mutagenic & anti-carcinogenic effect, modulation of immune response, anti-diarrheal and anti-allergenic reactions (Sandholm et al. 2002). However, variations exist in outcome of such investigations under different approaches that have been adopted to evaluate the functionality. The establishment of associated health benefits by consuming a certain probiotic dairy products through in-vivo assays is critical for the further success of this segment of functional foods. It has prompted newer initiatives at various forums to develop a guideline for efficacious investigations of probiotics for bringing the synergy among agencies involved and create confidence among consumers. The aim of the present chapter is to review the important group of probiotic microorganisms that have potential to be utilized for development of novel dairy foods including fermented milks, yoghurt, cheese, ice cream, composite dairy foods etc. The innovations that have been attempted to enhance the survivability probiotics across the value chain is dealt in depth. The review will also focus on mechanisms that are associated with therapeutic effects of probiotics with special reference to dairy products and their validation through In-vivo investigations.

6. Issues related to technological aspect of novel dairy foods

Designing of suitable diet with desired nutrients and pharmacologically-active components to meet the diverse needs of consumers is quite a daunting task. The healing power of milk nutrients is known for centuries and recent scientific investigations have proved the disease preventing or alleviating properties of milk nutrients. Several species of Lactic acid bacteria (LAB) assist in maintenance and improvement of gut health besides providing several other health benefits. It has been exploited all over the world for the development of probiotic dairy foods. Now the time has come when characterized indigenous probiotic microflora with proven technological and therapeutic attributes should be made available for the manufacture of novel probiotic dairy product. Although, probiotics have already started cementing their place in global dairy market, but many mysteries and health claims associated with probiotics needs to be addressed carefully.

Further, milk mining for the isolation of such bioactive molecules through appropriate technological interventions has gathered momentum in recent past. Traditional dairy products (TDP) which comprise the largest segment of processed dairy products also needs a face-lift through innovations in formulations and processes to reduce the fat and sugar levels without affecting their consumer acceptability. Newer ingredients and processes like membrane processing, high pressure processing (HPP) and supercritical fluid extraction (SCE), offer newer opportunities in delivering “wholesome” dairy products. Delivery of bioactive components in dairy products and its stability during the entire value chain is another major challenge. Various interventions including microencapsulation and nanotechnological could be the next important research area in coming days. Consumer acceptability of functional dairy foods will largely depends on their excellent sensory profile, validated health benefits and also their cost effectiveness. The R&D efforts in these areas will help the Indian food industry to deliver nutritional and therapeutic products to consumers and also diversify their product profile to sustain.
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<tr>
<th>Dairy based Functional Foods</th>
<th>Manufacturer</th>
<th>Applications</th>
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<tbody>
<tr>
<td>Pepto Pro® (di- and tripeptiedes)</td>
<td>DSM, Switzerland</td>
<td>Sports drink</td>
</tr>
<tr>
<td>Insu Vital™ (extensively hydrolysed casein)</td>
<td>DSM, Switzerland</td>
<td>Stimulate the secretion of insulin</td>
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<td>Recaldent™ (casein phophopeptides)</td>
<td>Recaldent</td>
<td>Chewing gums and Gels for remineralisation to prevent tooth decay</td>
</tr>
<tr>
<td>AmealPeptide® (milk derived peptide mixture)</td>
<td>Calpis Ltd</td>
<td>Blood pressure management</td>
</tr>
<tr>
<td>Pedia Sure (hydrolysed, whey dominant protein source, 50% MCT, structured lipids and a prebiotic soluble fibre)</td>
<td>Abbott Nutrition, US</td>
<td>Sole-source or supplement nutrition (1-13 yrs)</td>
</tr>
<tr>
<td>Calcium Plus (Calcium fortified milk)</td>
<td>Candia, France</td>
<td>Improves bone health</td>
</tr>
<tr>
<td>PysiCAL (Calcium fortified milk)</td>
<td>PhysiCAL, Australia</td>
<td></td>
</tr>
<tr>
<td>Viva (Mg fortified milk)</td>
<td>Candia, France</td>
<td>Good for Cardiac patients</td>
</tr>
<tr>
<td>Magnesio (Mg fortified milk)</td>
<td>Lactalis, France</td>
<td></td>
</tr>
<tr>
<td>Heart Plus (Omega-3 fatty acid-fortified milk)</td>
<td>PB Foods, Australia</td>
<td>Improves cardio-vascular health</td>
</tr>
<tr>
<td>Nestle (Omega Omega-3 fatty acid-fortified milk)</td>
<td>Nestle, Malaysia</td>
<td></td>
</tr>
<tr>
<td>Natrel Omega-3</td>
<td>Natrel, Canada</td>
<td></td>
</tr>
<tr>
<td>Dawn-Omega fresh milk</td>
<td>Dawn, Ireland</td>
<td></td>
</tr>
<tr>
<td>Stolle Milk Alpha Milk with CPP and IgG</td>
<td>Stolle, Japan</td>
<td>Enhancement of immune function, better absorption of calcium</td>
</tr>
<tr>
<td>NaturLinea (Milk with Conjugated Linoleic Acid)</td>
<td>Asturina, Spain</td>
<td>Immune enhancer</td>
</tr>
<tr>
<td>Evoulus (Milk with peptides)</td>
<td>Valio</td>
<td>Anti-hypertensive</td>
</tr>
<tr>
<td>Activate (Bifidobacteria BBiT™), Probiotic dinking yoghurt</td>
<td>Meadow Fresh (NZ)</td>
<td>Provides strain specific health related benefits and improves the gut health</td>
</tr>
<tr>
<td>Bactive Probiotic</td>
<td>Danisco, Poland</td>
<td></td>
</tr>
<tr>
<td>Benecol (Emmi, Yoghurt with phytosterol)</td>
<td>Benecol</td>
<td>Anti-hypercholesteromic</td>
</tr>
<tr>
<td>Evolus (yoghurt with phytosterol)</td>
<td>Valio</td>
<td>Anti-CHD</td>
</tr>
<tr>
<td>Flora Pro-Activ</td>
<td>Uniliver, UK</td>
<td></td>
</tr>
<tr>
<td>Z E N (Yogurt with Mg)</td>
<td>Danone, Belgium</td>
<td>Improves cardiac health</td>
</tr>
<tr>
<td>Mother’s Choice (milk based infant formula with DHA and ARA)</td>
<td>PBM Products</td>
<td>For cognitive development</td>
</tr>
<tr>
<td>Unislim (Ice cream with Bb-12 and Inulin)</td>
<td>Silver Pail Dairy</td>
<td>Improves gut microflora</td>
</tr>
</tbody>
</table>
7. Validation and Safety Issues

Appropriate validation studies through in-vitro, in-vivo or clinical trials have always been a great concern in investigating the mechanisms associated with functional food consumption and also determining the safety and toxicity. The optimal levels of the majority of the biologically active components currently under investigation have yet to be determined. In addition, a number of animal studies show that some of the phytochemicals (e.g. allyl isothiocyanate) for their cancer-preventing properties have been shown to be carcinogenic at high concentrations. Designing of suitable animal and clinical investigations require multidisciplinary approaches including experts from diverse fields. The benefits and risks to individuals and populations as a whole must be weighed carefully when considering the widespread use of physiologically-active functional foods. Knowledge of toxicity of functional food components is crucial to decrease the risk: benefit ratio.

8. Conclusion

Mounting evidence supports the observation that functional foods containing physiologically active components may enhance health. It should be stressed, however, that functional foods are not a magic bullet or universal panacea for poor health habits. Moreover, diet is only one component of an overall life-style that can have an impact on health; other components include smoking, physical activity, and stress. Health-conscious consumers are increasingly seeking functional foods in an effort to control their own health and well-being. The field of functional food, however, is in its infancy. Claims about health benefits of functional foods must be based on sound scientific criteria.

9. Suggested Reading


Sharma, H., Nair,M. M. 2012. Nutraceuticals- Critical Supplements for Building a Healthy India. FICCI-Ernst-Young Knowledge Paper, 80 p

Extrusion: Novel Technology for Snack Foods

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Dairy Technology Division

1. Introduction

Snack is defined as a little meal that is eaten between the main meals of the day. It is intended to temporarily satisfy a person’s hunger until the next meal and at the same time provide a brief supply of energy needed by the body. Snack foods are designed to offer convenience in eating, preparation, packaging and storage. They comprise of a very large variety of items including potato chips, crackers, nuts and extruded snacks etc. Most of the manufactured snacks are high in calories and fat but low in protein, vitamins, and other nutrients that has attributed to the problem of obesity especially among children and thus snacks are often classified as “junk foods”. Snacks with increased nutritional value and protein content can be produced by adding high-quality protein sources including milk protein, legumes (peanut, soybean and cowpea) as well as fish, pork, beef, and chicken to the traditional starch base and using the extrusion process to combine the two. Extrusion cooking can also be used to bring down the level of anti-nutritional factors in snacks and improve its digestibility. Furthermore, extrusion cooking has the advantages of being time saving, energy efficient, no effluent generation and cost effective process.

According to a report by KPMG, the market value for extruded snacks in 2009 was $223.7 million which has reached $298.7 million in 2011. In India, the snack food market is quite large and diverse consisting of more than 1000 variants and about 300 types of savories. The growth rate in organized and unorganized sector is reported to be in the range of 15-20% and 8% respectively. Today snack foods are highly subjected to impulse buying and have gained popularity due to growing urban population, increase in number of nuclear families and working women, media penetration leading to attraction for novel food, and higher incomes. With growing concern among people for the diet health, nutrition and therapeutics, the food industry has been constantly trying to produce ‘lighter’ diet products. Use of functional ingredients and extrusion cooking are thus being increasingly used to add desirable sensory characteristics and nutritional functionality to a wide array of snacks.

2. Extrusion technology

Extrusion technology is a high temperature short time process whereby the material is modified through the unique combination of high temperature, pressure and shear forces (De Mesa et al., 2009). The process combines several unit operations including mixing, cooking, kneading, shearing, shaping, and forming. It causes certain changes in the food products like hydration of starches and proteins, homogenization, gelation, shearing, melting of fats, denaturation or re-orientation of proteins, plasticization and expansion of the food structure, inactivation of microbes, enzymes and many anti-nutritive factors in food (Bhattacharya and Prakash, 1994; Frame, 1994).

2.1 Food extruders

Food extruders (extrusion cookers) belong to the family of HTST (high temperature short time) equipment, capable of performing cooking tasks under high pressure at high temperatures and in short period of time.
Extruders come in a wide variety of size and with different methods of operation, but they can be mainly classified into two types: single and twin-screw extruders, according to their construction. The principle of operation is similar in both types. Feed material in granular form is fed into the extruder barrel having a steam jacket or is electrically heated. The screw then conveys the material, compresses and works it to transform the granular feed material into a plasticized mass. The food is then extruded through an interchangeable die and is cut after the die either by rotating knives to form a variety of shapes such as rods, spheres, doughnuts, tubes, strips or shells (Riaz, 2000; Brennan, 2006).

2.1.1. Single-screw extruders

A single screw extruder is designed with one screw to move the feed material through the barrel. The friction on the material by the cylindrical wall keeps it turning and moving with the screw. The typical single-screw extruder consists of three zones: feeding zone, kneading zone and cooking zone (Frame, 1994). Single-screw extruders can be categorized into low-shear extruders, high-shear extruders, cold forming extruders, high pressure forming extruders and collet extruders based on several different characteristics, i.e., extent of shear generated by these extruders, wet vs. dry, segmented vs. solid screw, and source of heat generation (Riaz, 2000).

- Low–shear extruders are moderate-shear machines with high compression screws and grooved barrels to enhance mixing. Heat can be applied to the barrel or screw to cook the product. Soft-moist foods and meat-like snacks can be made with these machines.
- High shear extruder aims to maximize mechanical energy input and is used in applications where heating is required. They are high-shear machines with screws for changing flight depth and/or screw pitch with high compression ratios, high temperatures, and various degrees of puffing (Linko et al., 1981).
- Cold forming extruders are low-shear machines with smooth barrels, deep flights, low screw speeds, originally used to work moistened flour and press it through a die with little cooking.
- High pressure forming extruders having grooved barrels and compressing screws are typically used to extrude pre-gelatinized cereal and other dough through dies to make pellets for subsequent drying and puffing or frying. Various cereals and fried snack foods can be made with these machines.
- Collet extruders are high-shear machines with grooved barrels and screws with multiple shallow flights that have been used for making puffed snacks from defatted corn grits. The temperature of relatively dry (12% moisture) ingredients is raised rapidly to over 175 ºC, and the starch is dextrinized and partially gelatinized. The resulting mass loses moisture and puffs immediately upon exit through a die to form a crisp, expanded curl or collet (Linko et al., 1981).

2.1.2 Twin-screw extruders

Twin-screw extruders differ from the single-screw extruder in terms of their processing capability and mechanical characteristics, and are largely responsible for the increasing popularity of extrusion processing. The position of the screws in relation to one another and their direction of rotation are often used to categorize twin-screw extruders. It can have intermeshing screws in which the flights of one screw engage the other, or they can have non-intermeshing screws in which the threads of the screws do not engage one another, allowing one screw to turn without interfering with the other. Twin-screw extruders may have co-rotating or counter-rotating screws. Both co-rotating and counter-
rotating extruders can have fully, partially or non-intermeshing screws (Harper, 1987; Frame, 1994; Brennan, 2006).

2.2 Functions of an extrusion cooker

The conditions generated by the extruder permit the performance of many functions that allow it to be used for a wide range of food, feed, and industrial applications. Some of these functions are:

• Mixing - A variety of screws are available, which can cause the desired amount of mixing action in the extruder barrel.
• Grinding - Ingredients can be ground in the extruder barrel during processing.
• Shearing - A special configuration within the extruder barrel can create the desired shearing action for a particular product.
• Thermal cooking - The desired cooking effect can be achieved in the extruder.
• Gelatinisation - Extrusion cooking improves starch gelatinisation.
• Protein denaturation - Animal and plant protein can be denatured in extrusion cooking.
• Expansion - Product density (i.e., floating and sinking) can be controlled by extruder operation conditions and configuration.
• Shaping - An extruder can be made to produce any desired shape of the product by changing a die at the end of the extruder barrel (Riaz, 2000).

2.3 Advantages of extrusion cooking

Extruders are used widely in the manufacture of a number of foods, because of their capabilities to cook, mix, texturize and shape food products under conditions of continuous production and at low cost. Other advantages over traditional cooking methods are as under:

• Versatility – A variety of foods can be extruded using the same equipment (Guy, 2001).
• High product quality – The HTST process and the shear stress kills microorganisms with minimal nutrient degradation (Williams et al., 1977; Harper, 1979; Bulut et al., 1999).
• Product characteristics - A variety of shapes, colours and appearances can be produced, which is not so easily possible by other production methods (Riaz, 2000).
• Improves digestion – Legume crops, particularly peas and beans, are important feed ingredients, but difficult to digest; however, the nutritional value of these crops are increased after they have been extruded (Gilbert, 1998).
• Lower processing cost and higher productivity compared to other cooking and forming processes (Guy, 2001).

2.4 Application of extrusion technology in food processing

2.4.1 Ready to eat breakfast cereals (RTE)

RTE cereals are manufactured from mixtures of cereal flours and starches combined with small amounts of malt, fat, sugars, emulsifiers, and salt (Miller, R.C. 1994). Extrusion is one of many techniques to manufacture RTE cereals. Extrusion cooking has become the preferred technology because it accepts both cereal and starch ingredients, creates highly expanded products, produces a wide variety of shapes and textures, cooks and forms in a single processing step, and works at relatively low moistures at satisfactory cost.
Two types of extrusion-cooked breakfast cereals can thus be found on the market:

- Directly expanded extrusion-cooked breakfast cereals: Cereal flours and/or grits are cooked with ingredients and with very low moisture content (usually below 20%). The process may use single or twin-screw extruders, the configuration and operating characteristics of which generally lead to highly mechanical cooking.

- Pellet-to-flakes extrusion-cooked breakfast cereals: Cereal flours and/or grits are cooked with ingredients and at a moisture level in the range of 22–26%. They are usually processed in twin-screw extruders, the configuration and operating characteristics of which lead to a lower mechanical component of cooking, reinforcing the thermal component as opposed to the previous processing conditions.

Flow Chart for Development of Ready to eat breakfast cereals

2.4.2 Snack foods

Snacks contribute an important part of daily nutrient and calorie intake for many consumers. One advantage of extrusion cooking in snack food production is the ability to produce a wide range of finished products with the combination of cereals and dairy ingredients with minimum processing times using inexpensive raw materials. Extrusion enhances the nutritive value of products by minimizing the losses of nutrients such as protein, vitamins, etc. while giving rise to a new range of products.

The snack food processes using extrusion cooking are categorized as direct expanded extrudate, extruded pellets or half products of the third generation snacks which require a further processing step prior to consumption. Snack food technology of direct expanded, and shaped snacks is similar to RTE cereals processes, but normally performed at lower moistures, so that a higher energy input from mechanical sources occurs. In general, direct expanded products are made using high-shear extruders. Other categories of the
direct expanded foods are crisps or flat breads, which are produced using the twin-screw extruder technology. Also, twin-screw extruders can be used for cracker production. Co-extrusion is a process that combines two different extruded streams to obtain two-component products characterized by a dual texture and/or colour.

Ingredient Storage
- Premixing
- Moisture adjustment
- Blending
- Sieving
- Preconditioning (overnight)
- Ingredient Feeding
- Extrusion cooking
- Drying or Baking
- Coating
- Packaging

**Flow Chart for Development of expanded extruded snack**

2.4.3. *Pet foods and aquatic foods*

Pet foods, aquatic foods and other animal foods are important cereal-based products by extrusion cooking. They consist of a mixture of cereals, vegetable proteins, meat by-products, fat, flavors and vitamins. Pet foods represent the greatest volume of extruded products on the market, which include dry expanded and unexpanded semi moist products. Expansion of the final extruded product is an extrusion technology tool to control product density in the manufacture of the floating or sinking aquatic foods.

2.4.4. *Texturized vegetable protein*

Cross-linking reactions of the vegetable protein during extrusion cooking leads to form fibrous meat-like structure that can be used as meat substitute in a variety of foods.

2.4.5. *Confectionery products*

Twin-screw extruders are able to mix the material well, control temperatures of the heat sensitive materials and incorporate additional fat, milk, sugars, nuts, and other ingredients, which are used in the confectionery industry. Twin-screw extrusion technology can be used to process in one step the coating grade chocolate and the injection moulded or formed chocolate articles.
### Table 2.1: Examples of extruded foods*

<table>
<thead>
<tr>
<th>Type of product</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal–based products</td>
<td>Expanded snack foods</td>
</tr>
<tr>
<td></td>
<td>RTE and puffed breakfast cereals</td>
</tr>
<tr>
<td></td>
<td>Soup and beverage bases, instant drinks</td>
</tr>
<tr>
<td></td>
<td>Weaning foods</td>
</tr>
<tr>
<td></td>
<td>Pre-gelatinized and modified starches, dextrin</td>
</tr>
<tr>
<td></td>
<td>Crisp bread and croutons</td>
</tr>
<tr>
<td></td>
<td>Pasta products</td>
</tr>
<tr>
<td>Sugar-based products</td>
<td>Chewing gum</td>
</tr>
<tr>
<td></td>
<td>Liquorice</td>
</tr>
<tr>
<td></td>
<td>Toffee, caramel, peanut brittle</td>
</tr>
<tr>
<td></td>
<td>Fruit gums</td>
</tr>
<tr>
<td>Protein-based products</td>
<td>Texturized vegetable protein (TVP)</td>
</tr>
<tr>
<td></td>
<td>Semi-moist and expanded pet foods and protein supplements</td>
</tr>
<tr>
<td></td>
<td>Sausage products, frankfurters, hot dogs, Surimi</td>
</tr>
<tr>
<td></td>
<td>Caseinates</td>
</tr>
<tr>
<td></td>
<td>Processed cheese</td>
</tr>
</tbody>
</table>


### 3. Concluding remarks

Extrusion cooking has emerged as a versatile technology in food processing, one of its important applications being production of expanded snacks. Advantages of extrusion cooking include reductions of nutrient destruction, improvement in starch or protein digestibility and enhanced product characteristics and range.

### 4. Suggested Reading


