National Training
on
Advances in Production, Functional, Rheological & Quality Aspects of Traditional Indian Dairy Products

October 8 - 28, 2013

Organized by
Dairy Technology Division
Under the aegis of
Centre of Advanced Faculty Training in Dairy Processing

National Dairy Research Institute (Deemed University), ICAR
Karnal - 132 001
Training Manual

Advances in Production, Functional, Rheological and Quality Aspects of Traditional Indian Dairy Products

28th National Training

October 08 - 28, 2013

Dr. Kaushik Khamrui
Course Director

Mr. Devaraja, H.C
Mr. Yogesh Khetra
Dr. Surajit Mandal
Course Co-Directors

Centre of Advanced Faculty Training in Dairy Processing
Dairy Technology Division
National Dairy Research Institute
(Deemed University)
(Indian Council of Agricultural Research)
Karnal 132001 (Haryana), India
Published by: Dr V.K. Gupta
Head, Dairy Technology Division &
Director, CAFT in Dairy Processing

Cover Page Designed by: Mr. Devaraja H.C.
Mr. Yogesh Khetra
Dr. Kaushik Khamrui

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Mr. Sathish Kumar M.H.
Mr. Yogesh Khetra
Mr. Devaraja, H.C
Dr. Surajit Mandal
COMMITTEES FOR ORGANIZATION OF THE 28th NATIONAL TRAINING
ON
ADVANCES IN PRODUCTION, FUNCTIONAL, RHEOLOGICAL AND QUALITY ASPECTS OF TRADITIONAL INDIAN DAIRY PRODUCTS

(8th October to 28th October, 2013)

Organizing Committee

Dr. V.K. Gupta, Director, CAFT
Dr. S. K. Kanawjia PS, DT
Dr. Kaushik Khamrui, SS, DT
Dr. S. Mandal, Sci, DM
Mr. Yogesh Khetra, Sci, DT
Mr. Devaraja, H.C. Sci, DT

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Dr. Latha Sabikhi
Mr. Sathish Kumar M.H.

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Mr. Devaraja, H.C.

Purchase Committee:
Dr. P.N. Raju
Mr. G.S. Meena
Mr. Sathish Kumar M.H.
Foreword

From the time immemorial, Traditional Indian Dairy Products have been the integral part of India’s culinary and cultural heritage. Traditional dairy products were developed to preserve the nutritional goodness of milk and to extend shelf-life under relatively high ambient temperature. It is estimated that nearly half of the annual production of milk is converted to a variety of traditional milk products viz., paneer, khoa, burfi, pada, ghee, dhana, dahi, shrikhand, rasogolla, sandesh, etc. Each of the products has its distinctive wisdom as it evolved through the ages, continue to surprise the gastronome even today.

In the last decade this sector has received due attention from the scientists and work has been carried out to standardize their manufacturing techniques, characterization of the quality requirements and enhancement of their shelf life. Development of commercial methods for the manufacture of khoa, gulab jamun, dahi, shrikhand, pada, ghee, rasogolla, etc are the result of concerted efforts of scientific manpower. In recent years the technologies of many convenience and ready to use traditional dairy products with extended shelf life e.g., gujab jamun mix powder, kulfie mix powder, raso malai and basundi mixes and kheer powder have been developed. UHT and retort processing are now being adopted by the organized sector for production of lassi, basundi, chhach, kheer, long life paneer, milkcake, etc. Technologies for many functional milk sweets enriched with pre and probiotics, fiber, vitamin and natural phyto-chemical enriched products with health attributes has also been developed.

The training course on “Advances in Production, Functional, Rheological and Quality Aspects of Traditional Indian Dairy Products” has been designed to comprehensively cover the various production, functional, rheological and quality aspects of traditional Indian dairy products. It will include theory as well as practical sessions by academic personnel from NDRI and invited experts from leading dairy industry and academia. The authors have also compiled all the recent developments on the chosen topics in the form of a compendium. This compendium will not be only of great use to the participants for dissemination the knowledge but also will act as a stimulant to open new avenues to the research works in the area of traditional dairy products. I wish the training course a great success.

(A.K. Srivastava)
ACKNOWLEDGEMENT

The Indian Council of Agricultural Research accorded Dairy Technology Division of NDRI as a centre of Excellence in Dairy Technology for its Centre of Advanced Studies programme in the year 1996. The centre has recently been renamed as ‘Centre of Advanced Faculty Training (CAFT) in Dairy Processing’ by the ICAR. In the past, 27 training programmes in different areas of dairy processing have been successfully organised under CAFT Programme. This is 28th course on “Advances in production, functional, rheological and quality aspects of traditional Indian dairy products” conducted during October 8-28, 2013. This course will be highly useful for the participating researchers and teachers of Agricultural Universities, national and other academic institutions in further updating their knowledge in the area of traditional Indian dairy products.

We express our gratitude to the Indian Council of Agricultural Research for awarding CAFT in Dairy Processing to NDRI, Karnal. We take this opportunity to thank Dr. Kusumakar Sharma, ADG (HRD) for approving this short course 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. G.R. Patil, Joint Director (Academics), NDRI, Karnal, in this CAFT programme is gratefully acknowledged.

Dr. Kaushik Khamrui, Senior Scientist and Course Director deserves special mention for his diligent efforts that made the initiation of this programme a success. He has been very ably supported by Dr. Surajit Mandal, Mr. Devaraja H.C. and Mr. Yogesh Khetra, (Scientists, Course Co-directors) in this endeavour. Compilation of various lectures into a compendium, its editing and formatting is a stupendous job. The role of Ms Ritika Puri, Ms Shilpi Gupta and Ms Sakshi Narula for their valuable assistance in formatting the manuscripts of the lectures for the compendium is appreciable.

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 are highly indebted to the guest speakers who contributed the lecture material well in time and traveled to Karnal to share their valuable expertise with the participants. We also thank M/s Moderns Dairies, Karnal for permitting the participants to visit their processing facility. Further, the contribution of Chairmen and members of different committees for smooth organization of this training programme is highly appreciable. We are grateful to the technical, ministerial and supporting staff of Dairy Technology Division for their contribution to day-to-day activities of this CAFT course.

Date: October 8, 2013

(V. K. Gupta)
<table>
<thead>
<tr>
<th></th>
<th>Name</th>
<th>Position</th>
<th>Institution</th>
<th>Address</th>
<th>Email</th>
<th>Mob</th>
</tr>
</thead>
<tbody>
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### 08 – 10 – 2013 (Tuesday)

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<th>Time</th>
<th>Activity</th>
<th>Presenter</th>
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<tbody>
<tr>
<td>09:30 – 10:00AM</td>
<td>Registration</td>
<td>Dr. Latha Sabikhi</td>
</tr>
<tr>
<td>10:00 – 11:30AM</td>
<td>Inauguration of the course</td>
<td>Dr. Kaushik Khamrui</td>
</tr>
<tr>
<td>11:30 – 12:00AM</td>
<td>Tea break</td>
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<tr>
<td>10:45 – 11:15AM</td>
<td>Visit to Experimental Dairy</td>
<td>Sh. S.K. Kharb</td>
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<tr>
<td>12:15 – 01:00AM</td>
<td>Visit to Livestock research centre</td>
<td>Sh. S.K. Kharb</td>
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<tr>
<td>01:00 – 02:00PM</td>
<td>Lunch</td>
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<tr>
<td>02:00 – 02:30PM</td>
<td>Visit to ATIC centre</td>
<td>Sh. Rajender Kumar</td>
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<td>02:30 – 03:00PM</td>
<td>Visit to National Library on dairying</td>
<td>Sh. Rajender Kumar</td>
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<td>03:00 – 03:30PM</td>
<td>Tea Break</td>
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<td>03:30 – 05:00PM</td>
<td>Visit to DE, DT, DM, DC laboratories</td>
<td>Mr. Devaraja H.C.</td>
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### 09 – 10 – 2013 (Wednesday)

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<tr>
<td>09:30 – 10:30AM</td>
<td>Current Scenario, Scope and challenges of Traditional Indian Dairy Products</td>
<td>Dr. G.R.Patil Joint Director, NDRI</td>
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<tr>
<td>10:30 – 11:30AM</td>
<td>Developments in processing of traditional dairy products</td>
<td>Dr. Kaushik Khamrui Sr. Scientist, DT Division</td>
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<tr>
<td>11:30 – 12:00AM</td>
<td>Tea Break</td>
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<tr>
<td>12:00 – 01:00PM</td>
<td>E.P.S producing lactic culture for Indian fermented dairy products</td>
<td>Dr. Pradeep behare Sci. DM division</td>
</tr>
<tr>
<td>01:00 – 02:00PM</td>
<td>Lunch Break</td>
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<tr>
<td>02:00 – 05:00PM</td>
<td>Ready-To-Reconstitute Formulations for Traditional Dairy Products (Theory and Practical)</td>
<td>Dr. Abdul Hussain Sci. DT Division</td>
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### 10 – 10 – 2013 (Thursday)

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<th>Time</th>
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<th>Presenter</th>
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<tbody>
<tr>
<td>09:30 – 10:30AM</td>
<td>Process innovation in fat rich dairy products</td>
<td>Mr. Sathish Kumar M.H. Scientist, DT Division</td>
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<tr>
<td>10:30 – 11:30AM</td>
<td>Technological Developments in Traditional Fermented Dairy Product</td>
<td>Dr. S.K Kanawjia PS, DT Division</td>
</tr>
<tr>
<td>11:30 – 12:00AM</td>
<td>Tea Break</td>
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<tr>
<td>12:00 – 01:00PM</td>
<td>Application of membrane processing in the Production of Indian dairy products</td>
<td>Dr. V.K Gupta PS, DT Division</td>
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<tr>
<td>01:00 – 02:00PM</td>
<td>Lunch Break</td>
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<tr>
<td>02:00 – 05:00PM</td>
<td>Membrane processing in the manufacture of heat coagulated dairy products (Practical)</td>
<td>Mr. G.S.Meena Sci., DT Division</td>
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### 11 – 10 – 2013 (Friday)

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<tr>
<td>09:30 – 10:30AM</td>
<td>Microbiological risk assessment of traditional milk products</td>
<td>Mr. Raghu H.V Scientist, DM Division</td>
</tr>
<tr>
<td>10:30 – 11:30AM</td>
<td>Biopreservation of traditional dairy products</td>
<td>Dr. R.K Malik Head, DM Division</td>
</tr>
<tr>
<td>11:30 – 12:00AM</td>
<td>Tea Break</td>
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<tr>
<td>12:00 – 01:00PM</td>
<td>Food safety management system for Indian dairy products</td>
<td>Mr. Devaraja, H.C Scientist, DT Division</td>
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<tr>
<td>Time</td>
<td>Activity</td>
<td>Speaker/Instructor</td>
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<tr>
<td>01:00 – 02:00PM</td>
<td>Lunch Break</td>
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<tr>
<td>02:00 – 05:00PM</td>
<td>Fortification of milk with mineral and vitamins (Theory and Practical)</td>
<td>Dr. Sumit Arora&lt;br&gt;PS, DC Division</td>
</tr>
<tr>
<td>09:00 – 05:00PM</td>
<td>Visit to the Industry</td>
<td>Mr. Yogesh Khetra / Mr. G S Meena&lt;br&gt;Scientist, DT Division</td>
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<tr>
<td><strong>12 – 10 – 2013 (Saturday)</strong></td>
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<tr>
<td>09:30 – 10:30AM</td>
<td>Fortification of Indian dairy products with functional ingredients</td>
<td>Dr. Latha Sabikhi&lt;br&gt;PS, DT Division</td>
</tr>
<tr>
<td>10:30 – 11:30AM</td>
<td>Application of SSHE for mechanized production of Indian dairy products</td>
<td>Dr. A.K Dodeja&lt;br&gt;Head, DE Division</td>
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<tr>
<td>11:30 – 12:00AM</td>
<td>Tea Break</td>
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<tr>
<td>12:00 – 01:00PM</td>
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<td>Dr. Rajan Sharma&lt;br&gt;Sr. Scientist, DC Division</td>
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<td>Dr. Surajit Mandal&lt;br&gt;Scientist, DM Division</td>
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<td>09:30 – 11:30AM</td>
<td>Application of Artificial Neural Network (ANN) in Food Processing and sensory evaluation</td>
<td>Dr. A.P. Ruhil&lt;br&gt;Guest Faculty, PS, ASRB</td>
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<td>Dr. Bimlesh Mann&lt;br&gt;PS, DC Division</td>
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<td>Dr. Latha Sabikhi&lt;br&gt;PS, DT Division</td>
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<td>12:00 – 01:00PM</td>
<td>Sensory Evaluation of Milk and Milk Products: Requirements and Technique</td>
<td>Dr. Kaushik Khamrui&lt;br&gt;Sr. Scientist, DT Division</td>
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<td>Technological aspects of composite traditional dairy foods (Theory and Practical)</td>
<td>Dr. A.K Singh&lt;br&gt;Sr. Scientist, DT Division</td>
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<td>Dairy products for the lactose intolerant population with special emphasis on Traditional Indian Dairy Products</td>
<td>Mr. Sathish Kumar M.H.&lt;br&gt;Scientist, DT Division</td>
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<td>Sensory Evaluation of Milk and Milk Products: Requirements and Technique</td>
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<tr>
<td>12:00 – 01:00PM</td>
<td>Technological aspects of traditional dairy beverages (Theory and Practical)</td>
<td>Mr. Yogesh Khetra&lt;br&gt;Scientist, DT Division</td>
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<tr>
<td>02:00 - 05:00PM</td>
<td>Technological aspects of traditional dairy beverages (Practical)</td>
<td>Mr. Yogesh Khetra&lt;br&gt;Scientist, DT Division</td>
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<td>12:00 – 01:00PM</td>
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<td>Dr. Meena Malik</td>
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<td>Process development for extended shelf life paneer with enhanced health attributes</td>
<td>Mr. Devaraja, H.C. Scientist, DT Division</td>
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<td>Developments in sensory evaluation techniques of traditional dairy products (Theory and Practical)</td>
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<td>Mr. Abdul Hussain Sci, DT Division</td>
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Indian traditional milk products have played a significant role in the economic, social, religious and nutritional well being of our people since time immemorial. It is estimated that about 50 to 55 per cent of milk produced is converted by the traditional sector (halwais) into variety of Indian traditional milk products, using processes such as heat and acid coagulation, heat desiccation, and fermentation. Market size of Indian traditional milk products is estimated at more than Rs. 100,000 crores with an annual growth estimated at Rs. 5,000 crores. This fact underlines the significance of Indian milk sweets in the national economy. In view of the growing awareness towards the safety aspects of milk based sweets in India, the consumer shall prefer to buy these products from the organized sector. Despite the widespread popularity and acceptability of traditional milk products in the Indian market, the organized sector has so far not been able to tap into this market potential for many reasons such as lack of published literature on their technology, inadequacy of appropriate technologies for their commercial production, inadequacy of appropriate packaging materials and labeling to take care of new pattern in consumer demand, low keeping quality and a lack of quality assurance systems. Data from the national sample survey revealed the rising trend in the monthly per capita expenditure on milk and milk products. Interestingly, such expenditure in rural areas of the Northern India is usually higher (20-43%) than in urban areas. Recently a few organized dairy sectors have started the production of traditional milk products on a commercial scale but their impact has been limited. While many new innovations have been made recently to modernize this sector, it is necessary to look into short, medium and long term strategies to develop core technological strengths within our industry for envisioning a developed indigenous dairy products sector. A vision for this sector is only possible through identifying such core strengths and building on them.

**Current status**

*Mechanization of manufacture of traditional dairy products*

Deep rooted tradition offer a considerable scope for organizing and channeling the amount of milk going for conversion into Indian milk sweets. The major strength of the Indian milk sweets sector is the mass appeal enjoyed by such a wide variety of products. The market for these products far exceeds that for western dairy products. Their operating margins are also much higher, mainly due to lower raw material cost. It is estimated that the raw material costs of Shrikhand, Rasogolla, Gulabjamun, Khoa sweets (Peda, Burfi, Kalakand), Sandesh and Paneer is 29, 33, 34, 35, and 65 per cent of the sale price, respectively. For western dairy products, comparative costs are relatively much higher varying from 7 - 80%. (Chandan et al., 2002). Their production and marketing can bring about a remarkable value addition to the extent of 200 per cent, as compared to only 50 percent obtained by western dairy products. They can do wonders for organized dairy sector to better its prospects of financial stability and steady growth.

Increasing demand for these products present a great opportunity for the organized dairies in the country to modernize and scale-up the production. The expanding business prospects provided by these products and their accompanying value-addition call for a thorough study of this sector. There is
a need to look into various issues and accordingly re-evaluate and re-engineer ourselves to modernize traditional dairy products sector.

In order to overcome the inherent disadvantages associated with conventional methods of manufacture of traditional dairy products such as inefficient use of energy, poor hygiene and sanitation, non-uniform product quality, fatigue on the operator, etc; attempts have been made to develop batch, semi-continuous, and continuous equipments for the manufacture of these products. The first attempt to develop semi-continuous Khoa-making machine was made by Banerjee et al. (1968) which was followed by batch type semi-mechanized scraped surface heat exchanger developed by More (1989-90); batch type mechanical conical process vat developed by Agrawala (1987) and scraped surface continuous Khoa making machines developed by NDDB (Punjanath et al., 1990), Dodeja et al. (1992) and Christie and Shah (1992). Some of these machines are already in commercial use in some dairies. The contherm-convap scraped surface heat exchanger system developed by ALFA-Laval is also being commercially used for the manufacture of Khoa. Recently, NDRI has developed a three stage SSHE for manufacture of khoa, which produces khoa as good as the traditional process.

Successful attempts have also been made to mechanize the methods of manufacture of Khoa based sweets. Palit and Dharam Pal (1998-99) developed mechanized manufacture of Burfi involving Khoa-making by continuous machine followed by kneading and heating Khoa-sugar mixture in Stephen kettle. The Sagar Dairy, Baroda manufactures kesar Peda by adopting a large-scale mechanized process which involves manufacture of Khoa using continuous machine, heating Khoa -sugar mixture in planetary mixer, cooling, mechanical forming of Peda and packaging. Recently, Londhe (2006) standardized method of manufacture of boown (Mathura) peda for industrial application. Similarly, Gulabjamuns are being manufactured commercially using Khoa portioning and ball forming machines followed by deep fat frying and sugar syrup soaking lines. (Banerjee, 1997). Pal et al. (2005) developed a method for large scale manufacture of rabri using thin film scraped surface heat exchanger.

Aneja et al. (1977) developed a prototype continuous Chhana making machine involving tubular heat exchanger, acid injection chamber, holding coil and strainer. A process has been developed on similar principle for the mechanized production of Chhana at IIT, Kharagpur, which involves indirect heating of milk in a tubular heat exchanger to 95ºC, cooling to 70 ºC, continuous coagulation with hot citric acid (70ºC) in a vertical tube, holding milk-acid mixture to permit complete coagulation, separation of whey in a continuous flow employing double wall basket centrifuge and chilling to 4º C by directly spraying chilled water on the layer of Chhana (Singh, 1994).

Considerable research has been carried out for optimization of the process for the manufacture of Paneer (Mathur et al., 1993). A prototype machine for continuous manufacture of Paneer has been developed recently at NDRI (Agrawala et al., 2001).

Developments have also been made in mechanization of Chhana-based sweets. Kumar et al. (1997) designed a screw conveyor for kneading of Chhana and a cutter provided at the exit split the Chhana into lumps of 10 g each. The lumps are made to fall on a spinning disc and stationary disc above, which converts lumps of Chhana into round balls. Another machine has recently been developed at NDRI, Karnal, which involves kneading of Chhana using screw conveyor, portioning Chhana into lump of 10 g each with a cutting device, and ball formation in a revolving cylinder (Chaudhary et al., 2001). Kumar (1998) developed a single screw vented extruder for continuous production of Sandesh.

A fully mechanized/continuous process has also been developed for industrial production of Shrikhand (Aneja and Vyas, 1983). In this process, Chakka is prepared by separating the whey from
skim milk dahi employing 28” dia. basket centrifuge at 1100 rpm. The resultant Chakka, sugar and plastic cream are then mixed in a planetary mixer.

NDRI has perfected continuous equipment for manufacture of 500 kg ghee per hour (Abhichandani, 1997). This equipment is integrated with an efficient butter melter developed at NDRI.

**Application of membrane technology in manufacture of traditional dairy products**

A process has been developed for the manufacture of Khoa using reverse osmosis (RO) (Dharam Pal and Cheryan, 1987). Khoa manufactured from RO concentrated whole milk (31% TS) was comparable in flavor and texture to conventional product, with net energy saving of 335 to 430 kcal/kg of milk.

Preparation of good quality Chhana using skim milk ultrafiltered/diafiltered retentate and plastic cream has been reported (Sharma and Reuter, 1991). Heat treated (92°C for 5 min) skim milk is subjected to ultrafiltration followed by diafiltration (23 % TS) and the resultant retentate is mixed with plastic cream and mixture heated to 90°C for 5 min and coagulated with lactic acid to develop soft coagulum. The granular mass is pressed to remove free moisture, yielding Chhana. The yield of the product is 18-19 % extra due to recovery of whey proteins.

Production of good quality Paneer using ultrafiltration has been reported by Sachdeva et al. (1993). The process offers advantages like access to mechanization, uniform quality, improved shelf life, increased yield, and nutritionally better product. The process consists of ultrafiltration of heat-treated milk, cold acidification of retentate (40%TS), packaging in containers, and texturization by microwave heating. An innovative approach employing in-package sterilization of acidified UF retentate resulting in in-package coagulation and texturization was developed by Rao, (1991). The process yields long-life Paneer-type product with three-month shelf life at room temperature.

Adoption of membrane filtration process for manufacture of Chakka and Shrikhand results in high product yield. Sharma and Reuter (1992) developed a process for production of Chakka and Shrikhand using ultrafiltration technique. The process consisted of agitation of Dahi at slow speed, heating to 60-62°C for 5 min., ultrafiltration at 50°C, pressing of retentate to get Chakka and then mixing with sugar in planetary mixer. Sachdeva et al. (1994) attempted the manufacture of Chakka by reverse osmosis which involved heat treatment (90°C for 5 min.) to RO concentrate, cooling to 22°C, inoculation with 20% mixed lactic culture, incubation for 18 hrs and then removal of whey by filtration to get Chakka. Increased yield, higher solid recovery, reduced processing time, access to mechanization and alleviation of whey disposal problem were claimed as major advantages of the process. Kumar et al. (2005) reported manufacture of improved quality of chhana from diafiltered UF cow milk. An innovative new approach of adding coagulant to the retentate followed by heating to 60°C produced soft chhana suitable for manufacture of chhana based sweets.

**Developments in preservation of traditional dairy products**

The short shelf life of the traditional dairy products is the major limitation in organized marketing of these products. The conventional preservation techniques such as sterilization, freezing, etc. cannot be used for traditional dairy products due to their adverse effects on sensory and textural quality. This calls for application of newer concepts of food preservation such as hurdle technology, biopreservation, modified atmospheric storage, etc.

**Hurdle Technology**

The microbial stability and safety of most traditional foods is based on a combination of several preservation factors, called hurdles, which microorganisms present in food are unable to overcome.
These “hurdles” include water activity, pH, heat treatment, sugar, salt, redox potential, preservatives, etc. Hurdle technology involves optimization of 3 or more hurdles so that shelf life and the microbial safety is extended without adversely affecting overall quality of the product. The advantages of hurdle technology are: (i) the sensory and nutritional characteristics of food remain close to fresh/natural ones (ii) less energy consumption (iii) autosterilization of foods is observed during storage, and (iv) less susceptible to non-enzymatic browning and lipid oxidation. The first ever-successful application of hurdle technology in India was made in author’s laboratory for preservation of ready-to-eat Paneer curry (Rao and Patil, 1999). It involved optimization of water activity, pH, extent of heat treatment and level of preservatives to obtain shelf-stable product. The product has a shelf life of one month. Recently, application of hurdle technology in preservation of Paneer (Yadav and Sanyal, 1999) and heat coagulated colostrums milk (Premaralli, et al. 1999) has also been reported. The work on preservation to Burfi and milk cake using hurdle technology is in progress at NDRI.

Bio-preservation

Another emerging technology for preservation of perishable foods is “biopreservation”. It refers to extended shelf life and enhanced safety of foods using their natural or controlled microflora and/or their antimicrobial products. The lactic acid bacteria synthesize variety of inhibitory substances including bacteriocins or bacteriocidal proteins. Currently large-scale attempts on application of natural antimicrobials for food preservation are being carried out. Nisin was the first recognized antimicrobial substance produced by lactic streptococci that has realized commercial application in food preservation. Use of nisin in extension of shelf life of Khoa, Lassi, and sterilized Kheer has been reported (Salahuddin, 2002).

Many other bacteriocins produced by Lactobacillus spp. such as lactocin 27, helveticin J, Lactocidin, Plantaricin A & B, Sakacin – A, brevicin, Pediocin PA-I, pediocin AcH, Pediocin-A, Leucocin demonstrate broad range of antagonistic activity against many spoilage organisms. These bacteriocins need to be exploited for preservation of traditional dairy products. They will be particularly effective when used in combination with hurdle technology.

Osmotic dehydration

The concentration of food products by means of product immersion in a hypertonic solution is known as osmotic dehydration. This process has received considerable attention in recent years because of potential industrial application. Compared to air drying or freeze drying, osmotic dehydration is easier as less energy consuming because of removal of water occurs without a phase change. As the food product dehydrated by osmosis is not subjected to high temperatures for extended periods, the heat damage is also minimized. The technology has great potential in near future for dehydration of indigenous dairy products. Successful attempts have been made at author’s laboratory to dehydrate rasogolla and paneer using this technology.

Individual quick freezing (IQF) process

IQF process is another technology, which can be used for extending the shelf life of the traditional dairy products. IQF is a continuous process in which the product moving on the belt is exposed to a blast of extremely cold air freezing it in a matter of seconds. This serves two purposes – there is no time for the product to deteriorate and because it is frozen instantly the pieces do not stick to each other. Thus there are no clumps or blocks and one can take out even one individual piece without having to defrost or cut the frozen product. The great advantage of IQF is that the product reverts
practically to its original fresh state when used for consumption. The technology will be useful for preservation of paneer, rasogulla, gulabjamun, etc.

**Intermediate moisture products**

Reducing water activity of the food product to the intermediate moisture range is a well-known method of food preservation. This technique has recently been applied successfully at NDRI to preserve paneer cubes (Surinder Kumar, 2003). The intermediate moisture paneer has a shelf life of 4 months at room temperature and can be reconstituted within five minutes.

**Convenience traditional dairy products**

The changing life-styles and increased purchasing power especially among urban population has necessitated the research efforts for formulating ready-to use traditional milk products with added convenience, enhanced shelf life, added nutritive value, and with attractive packaging. Recently, number of such convenience products viz. Khoa, powder, Kulfi mix, Gulabjamun mix, Rasogolla mix, Burfi mix, Chhana powder, instant rice Kheer mix, Makhana kheer mix, Shrikhand powder, Lassi powder, dried carrot milk food mix, ready-to-eat Paneer curry, Chakka powder, Kadhi mix, Palada mix, Rasmali mix, basundi mix, long life paneer, long-life milk cake, etc. have been developed at NDRI and elsewhere, some of which are already being manufactured commercially.

**Functional traditional dairy products**

**Probiotic Traditional Dairy Products**

“Probiotic, food products in generals and “probiotic “ organism in particular are in the center of current R & D activities all over the world. “Functional foods” segment that is registering a steady and consistent growth at present, among processed food products, gathered the momentum primarily from the scientific investigations based on “probiotic” food products.

Industrial interest in developing probiotics and probiotic functional foods is thriving, driven largely by the market potential for foods that target general health or well being. NDRI has made some progress in this area by developing probiotic dahi, lassi, probiotic aloe vera lassi, and probiotic cheese. There is possibility of developing other milk based fermented traditional dairy products such as probiotic shrikand and Rabadi – a milk-cereal based fermented product.

**Fat-replacement in Indigenous Dairy Products**

High fat consumption has been linked to several chronic diseases including cardiovascular diseases, obesity and certain forms of cancer. Nutrition experts recommend a total fat intake of less than 30 per cent of total daily calories. These dietary recommendations are one reason for the increasing demand for lower fat food products of the world market has been flooded with the food products carrying the labels "low fat", 'no fat' or 'reduced fat'. Fat mimics or fat substitutes are normally used to produce low-fat foods, fat mimics are substances that help replace the mouthfeel of fat but cannot substitute for fat on a gram for gram basis and cannot be used for applications involving frying. Substances whose physical or thermal properties resemble fat are termed as fat substitutes and can replace fat on a gram-for gram basis and can also be used for frying applications.

Low-fat cheese, processed cheese, cultured products, frozen desserts, butters and spreads have been successfully developed using commercially available fat mimics/replacers. Using similar technique several low fat varieties of traditional dairy products can be developed. An attempt has been made to develop low fat burfi at NDRI, Karnal (Prabha, 2006).
A Heart-Healthy Opportunity

With the functional food market abuzz about the heart-health benefits of plant sterols, dairy foods formulators have excellent opportunity to develop variety of TDP with heart healthy benefit. Recently, a low cholesterol ghee has been developed at NDRI. The product has been commercialized and is now available in the market.

Arjuna ghee, with functionalities like resistance against heart diseases and blood pressure regulating properties was developed at NDRI, Karnal. The developed ghee was found sensorily similar to the market ghee. It had overall acceptability score of 85.1 compared to the control (90.84). The Arjuna ghee was found to be 4 times more stable to oxidative deterioration as compared to control ghee. This is due to the fact that Arjuna extract contains several antioxidants like polyphenols, terpenoids in addition to phytosterol, which are beneficial in case of Cardio-vascular Diseases (CVD), high blood pressure and to boost up our immune system.

Dietetic Indigenous 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 nonnutritive sweeteners have been witnessed in the market. Low calorie sweeteners have become sugar alternatives to replace sucrose in a wide variety of dairy products. Kumar (2000) developed a low calorie lassi by using aspartame and reported that aspartame at a level of 0.08 % was required to replace 15 % of cane sugar in lassi.

The technology for the production of rasogolla, the most popular channa based Indian sweetmeat, was developed by Jayaprakash (2003) using sorbitol (40 %) and aspartame (0.08 %). Chetana, et al. (2004) developed gulabjamun, a popular khoa based sweet, using sorbitol. Burfi, another khoa based sweet delicacy was developed by completely replacing sugar using acesulfame-K (Yarrakula, 2006), aspartame (Muralidhar, 2006), saccharin (Narendra, 2006), sucralose (Singh, 2006), and sucralose and bulking agents (Prabha, 2006). Kalakand and flavored milk were developed using acesulfame-K (Yarrakula, 2006), aspartame (Muralidhar, 2006), saccharin (Narendra, 2006), and sucralose (Singh, 2006). The Indian counterpart for ice cream, kulfi was developed by Pandit (2004) using sorbitol (5.5 %), maltodextrin (4.26 %) and aspartame (742 ppm).

Indigenous Dairy Products Fortified With Dietary Fiber

Milk and most dairy products are devoid of dietary fiber. With the growing interest in dietary fiber and its health benefits, dairy industry has geared up for fortifying the dairy products with fiber. In India, there are few traditional dairy products that contain significant quantities of fiber e.g., Gajar-pak (carrot halwa), Giya-ka-halwa (bottle gourd halwa), Doda-burfi, and Kaju-burfi. Traditionally made cereals-based milk desserts like kheer and dalia-dessert are other dairy food sources of dietary fiber in Indian diets (Patel and Arora, 2005). Recently, dahi (Chandrakant, 2002), lassi and other dairy products have been fortified with fruits and commercial dietary fibers to give the benefits of dietary fiber. Kantha (2005) developed a low fat paneer using soy fiber and inulin and reported that milk with 2.5 % fat and 0.56 % soy fiber or 1.8 % fat and 4.5 % inulin yielded a paneer similar to that prepared from full cream milk (6 % fat) in respect to sensory quality. Amul has launched a new variety of isabgol-enriched ice cream. Isabgol is the seed derived from Plantago ovata. Being a 'true dietary fibre', the isabgol husk is considered to be a natural laxative that aids easy bowel movement. Besides it is also known to possess serum cholesterol reducing properties (Mann and Singh, 2005).
Challenges, strategies and vision

Production of indigenous milk products by organized sector

Large-scale manufacture of these products in a hygienically safe manner with assured quality control and proper packaging will certainly do wonders for this sector not only in India but also abroad. Lead has initially been taken in this regard by NDDB’s Sugam dairy and since has been followed by Saber and Rajkot dairies in Gujarat; Warna dairy, Maharashtra; dairies in Madras, Bangalore, Hyderabad and Chittoor in South India, Mother dairy, Calcutta; KCMF, Trivendrum, COMFED, Patna; and many others. This organised production of indigenous dairy products, however, is miniscule as compared to total volume traded in the market. By 2020, we should shift at least 25% of production of Indigenous dairy products to the organized sector.

In spite of several innovative efforts made in the mechanization of manufacture of indigenous dairy products, adoption of these innovations by the industry is very limited. There may be several reasons for this. One reason may be our typical mindset. We appear to have lost faith in our abilities and ourselves. We seem to have a blind admiration of anything done outside our country and blindly believe whatever is foreign. We are by and large reluctant to adopt technologies developed locally.

It would also be pertinent at this stage to ask whether we need continuous systems or batch system when only a fraction of total ethnic products are processed in organized sector. Will it not be more appropriate to develop and promote batch type units so that mechanization of production in the small size units in the unorganized sector is effected thereby improving the hygienic quality of the products marketed by this sector?

The organized production does not necessarily mean large-scale production. We cannot afford to forget a large number of small and tiny manufacturing units, which are in the unorganized sector. A number of them have people with great innovative capabilities and basic skills. These talents need to be properly organized for hygienic production and marketing.

There is also a need to facilitate formation of consortia of dairy industry to fund research to (i) develop mechanized and energy efficient systems for manufacture and packaging of indigenous dairy products and (ii) develop value added indigenous dairy products for the future.

Packaging of traditional dairy products

Poor packaging of traditional dairy products is another big area, which should be strengthened. Most of these products particularly sweets are sold in open condition which is great source of contamination. Even products prepared by organized/large dairies, for example khoa and paneer are not properly packaged. No packaging system/machine is available for traditional milk sweets and the units available for non-dairy sweets are unsuitable for milk sweets. The methods of manufacture of many sweets also do not commensurate with the continuous packaging system. The appropriate and environmental friendly packaging materials are to be identified. Complete packaging systems that are in harmony with the production line will have to be adopted.

Training of small-scale operators

Most of the trade of Indian milk sweets is with the halwais and the small-scale operators. Most of them have art and skill of manufacturing varieties of indigenous dairy products. However, no attention is paid by them on quality of milk, hygienic handling, proper packaging and storage due to ignorance. The training of operators in this sector in hygienic handling and quality control aspects will go a long way in improving the quality of these products. The regional Agricultural Universities and Krishi Vigyan Kendras will have to play active role in training of small entrepreneurs.
Understanding basic characteristics of Indigenous dairy products

In order to modernize the Indian milk sweets sector, it is necessary to understand the basic characteristics of these products. The knowledge of these characteristics would contribute a great deal in design of equipments and standardizing scaled-up methods for manufacture of these products.

A variety of traditional dairy products are produced in India, most of which are region specific. Most of these products have been characterized for their chemical composition, sensory attributes and rheological and microbiological characteristics. Wide variation in composition of these products is observed due to variation to the method of manufacture, concentration ratio used, sugar level, type of milk (i.e cow, buffalo or mixed). There is a need to determine the consumers’ preference about the most desirable attribute of these products in different regions of the country so that the organized dairies may adopt the same.

Similarly, characterization of various food products on the basis of their rheology and microstructure forms the backbone of the scientific approach to product/ process development and of quality assurance in modern industrial practices. The current trends round the globe favour such studies to facilitate product description/specification for promoting process control and for international trade. At a juncture when the need for modernizing the manufacturing and marketing of traditional milk products is being emphasized in India, such rheological and electron microscopic studies would be sine qua non to obtain much needed information for product/process/equipment development. In the past few years, some work has been directed to study the rheology and microstructure of selected indigenous dairy products such as Paneer, Khoa, Rasogolla and Sandesh. It is also necessary to understand the kinetics of texture formation during manufacture of these products and the molecular level changes in the constituents of milk during processing. Any equipment designed without taking into consideration these basic aspects is less likely to be accepted by the industry as the product obtained using such equipment would lack the desirable texture.

Establishing national standards for indigenous dairy products:

Lack of quality/legal standards and quality assurance systems is one of the bottlenecks in improving the quality of these products. While legal standards for some of the milk sweets have been laid down, there is an urgent need to formulate the national standards for all the Indian sweets marketed in the country. There is also need to evolve the quality assurance system to meet the international standards of food hygiene and food safety.

Innovation in value added indigenous dairy products:

The markets of conventional indigenous products are increasingly getting overcrowded and our future success will depend on our ability to provide innovative products, which consumers want and need. Whatever the innovation - products, processing method or packaging - it should meet the real consumer need. We know today’s families want “grab-and-go” convenience. They are also concerned about nutrition and health. Different ages and demographics want different things. Therefore, investment at this level is essential if we are to respond rapidly to customers who are increasingly demanding new and different taste experiences from products that are also competitively priced. New variants of sweets can be developed. Indigenous dairy products containing health-promoting ingredients may be developed and promoted. Host of ingredients such as dietary fibre, cholesterol reducing phytosterols & phytostanols, minerals and vitamins, berries and cherries with its anthocyanins that prevent cancer etc. are available for value addition of traditional dairy products. Development of dietetic sweets is another area needing attention.
Innovation in marketing

Innovation in marketing is equally important. It is possible to popularize indigenous dairy delicacies through the fast food chains or franchising of some popular brands of Indian dairy delicacies may be promoted. Collecting market intelligence to inspire confidence among prospective entrepreneurs to take commercial production of traditional dairy products in India and abroad is also essential.

Epilogue

The Indian milk products enjoy mass appeal, give high profit margins and have high export potential. There is an urgent need to modernize this sector to produce high quality products with long shelf life. We need to generate basic data on these products which will help for designing of new equipments or for intelligent selection of existing food processing and packaging lines. Great scope also exists for improving the shelf life of milk sweets by employing newer preservation techniques. While lots of innovations have taken place recently, these innovations have not percolated to the actual users. Industry-R & D organization links need to be strengthened. Collaborative efforts of industry, unorganized sector, equipment manufacture and R & D institutions are required for all round development of this sector.

References


Application of Membrane Processing in the Production of Indian Dairy Products

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Introduction
The pressure driven membrane processes are based on the ability of semi-permeable membranes of appropriate physical and chemical nature to discriminate between molecules—primarily on the basis of size and to a lesser extent on shape and chemical composition. The main membrane systems in ascending order of pore size are: reverse osmosis (RO), nonofiltration (NF), ultrafiltration (UF) and microfiltration (MF). The distinction between RO, NF, UF and MF is somewhat arbitrary and has evolved with time and usage. In a broader sense, RO is essentially a dewatering technique, NF a demineralization process, UF a method for fractionation and MF a clarification process.

Membrane processes have many applications in the dairy industry and are increasingly being used because of several inherent advantages. Membrane processes can be carried out at ambient temperature. Thus, thermal degradation problems common to evaporation processes can be avoided resulting in better nutritional and functional properties of milk constituents. Further, these are continuous molecular separation processes that do not involve either a phase change or inter-phase mass transfer. Therefore, energy requirements of membranes processes are very low compared with other processes such as evaporation, freeze concentration, and freeze-drying. Further, easy, simple and economical operation, improved recovery of constituents and better yield of products are other advantages for which membrane processes are valued.

Application of reverse osmosis
RO is the most energy efficient dewatering process. Fluid milks and buttermilk can be partially concentrated economically using RO, particularly for the preparation of concentrated and dried products including indigenous dairy products like khoa, chakka, shrikhand, rabri, basundi and kheer. The economical levels of RO concentration for whole milk is up to 30% TS and for skim milk, 22% TS.

Khoa from RO concentrates
Khoa, an important indigenous Indian milk product, is presently manufactured on a small scale by continuous boiling of whole milk until a desirable solids concentration (65-70% total solids) is obtained. In recent years, several attempts have been made to develop new methods including the use of scraped surface heat kettles or heat exchangers for commercial production of khoa. The use of concentrated milk having up to 30% TS has produced khoa of highly satisfactory quality. The reverse osmosis, being energy effective process for pre-concentration of milk prior to the manufacture of khoa, has great potential in India. Khoa has been prepared from cow milk as well as buffalo milk by atmospheric boiling of RO retentates in a steam kettle (Gupta and Pal, 1994; Pal and Cheryan, 1987). The most important difference in control khoa and RO khoa was the higher moisture retention and lower free fat content in the later. Use of highly concentrated milk adversely affects the flavour quality. The process is conveniently amenable to continuous production of khoa from RO milk retentate using SSHE. Such process offers attractive energy saving in the initial concentration of milk. The energy consumption in RO concentration was estimated to be about 80 kcal/kg of milk for batch.
process and 25 kcal for continuous process, which brings about a net saving of 335 to 430 kcal/kg of milk.

**Chakka from RO concentrates**

Sachdeva *et al.* (1994) reported manufacture of ‘Chakka’ from milk concentrated by reverse osmosis (RO). Cow milk, standardised to fat : SNF ratio of 1 : 2.2 (12.5% TS), was pasteurised and concentrated (2.5 fold) using an RO plant. The concentrate was subjected to heat treatment of 90°C/5 min, cooled to 22°C, cultured at the rate of 2% with a mixed strain lactic culture and incubated for 18 hours. The coagulum thus obtained was filtered and a minimal amount of whey (4.5 lit./40 lit. of coagulum) having 18% TS was removed from it to get the chakka. Good quality shrikhand could be produced from RO chakka.

The RO chakka had 32.7% TS, fat 10.3%, 8.8% protein, 11.7% Lactose and 1.9% ash against the respective values for conventional chakka of 28.0%, 11.5%, 12.6%, 2.6% and 1.3%. The yield of RO Chakka was 35.5% as compared to 28.3% in case of conventional chakka. Increased yield, higher solids recovery, reduced processing time, increased throughput, access to mechanisation and alleviation of whey disposal problem are claimed as major advantages of this process.

**Application of nanofiltration**

Pal *et al.* (2002) and Sudhir (2002) reported that the inherent problem of salty taste and sandy texture in khoa could be overcome by nanofiltration of cow milk to 1.5 fold concentration before khoa manufacture. Dahi prepared from nanofiltered cow milk was also found to be superior to that of normal cow milk dahi.

**Application of ultrafiltration**

Ultrafiltration has a wide range of applications in the dairy industry. From milk, UF produces a permeate containing water, lactose, soluble minerals, non-protein nitrogen and water-soluble vitamins and a retentate in which proteins, fat and colloidal salts content increase in proportion to the amount of permeate removed. The process has also been used for the manufacture of several fermented dairy products like Yoghurt and Srikhand. UF retentate seems to be a highly promising base for chhana, rasogolla mix powder, long-life paneer. UF technology has also been applied to upgrade khoa manufacture from cow and buffalo milks.

**Chhana**

Preparation of good quality chhana using skim milk ultrafiltered-diafiltered retentate and plastic cream has been reported (Sharma and Reuter, 1991). Skim milk, heated to 95°C for 5 min., is ultrafiltered (26% TS). The retentate is diafiltered (23% TS) with equal amount of water to reduce lactose. For preparation of chhana, the retentate is mixed with plastic cream to a protein/fat ratio of 0.722. The mixture is heated to 85-90°C/5 min. and coagulated with dilute lactic acid to develop the characteristic grain. The granular mass is subsequently pressed to remove free moisture, yielding chhana. The process is reported to yield about 18-19 percent extra product and also no significant difference in flavour, body and texture and appearance compared to traditional method. High yield, easy automation and flexibility in operation are emphasized as advantages of this method for adoption for large-scale production.

Kumar *et al.* (2005) reported improved quality of UF chhana from cow milk. Cow skim milk was ultrafiltered and diafiltered to an optimum 23.88% TS. The required quantity of 63-65% fat fresh cream was then added to the UF retentate for standardization of fat. An innovative new approach i.e. addition of coagulant to UF retentate mixture at room temperature and then heating to coagulation
temperature, optimum being 60°C, resulted in production of desired softer *chhana* with higher moisture content, suitable for making sweets (*rasogolla* and *sandesh*), along with higher yield (12.92%) and higher total solid recovery (10.89%) than in traditional *chhana* and lesser total solid losses in whey compared to when UF *chhana* was prepared using traditional approach. Slow stirring (60-80 rpm) during heating and coagulation of UF retentate mixture yielded lower moisture (54.53%) content in *chhana*, compared to 56.93% moisture with rapid stirring (130-150 rpm). Standardized UF *chhana* met PFA standards and was comparable to traditional *chhana* organoleptically. *Rasogolla* and *sandesh*, prepared with modified process from UF *chhana*, scored "liked moderately" to "liked very much" on sensory evaluation.

Kumar (2006) standardized the manufacturing process of good quality *chhana* from a mixture of buffalo milk and sweet cream buttermilk by employing UF process. The standardized process gave higher yield (13.03%) and higher total solid recovery (11.49%) in UF *chhana* compared to the traditional process. The standardized UF *chhana* had 57.6% moisture and scored 7.5 for body and texture on 9-point Hedonic scale. The manufacturing process of optimum quality *rasogolla* and *sandesh* produced from UF *chhana* were also standardized. UF *rasogolla* & *sandesh* scored 7.7 & 8.17, respectively, for overall sensory acceptability on 9-point Hedonic scale.

**Rasogolla Mix Powder**

Manufacture of *rasogolla* is probably most difficult amongst all the milk-based delicacies. It requires lot of art and experience in addition to the right type of raw materials. The use of ultrafiltration process has been made in our endeavour to produce base for the *rasogolla* mix powder (Pal *et al*., 1994). Cow skim milk is ultrafiltered to about 3-fold concentration to achieve a product containing all the milk proteins and part of the minerals and lactose. To reduce the mineral and lactose level to almost the same level as in *chhana*, UF retentate has to be diafiltered. The pasteurised cream is added to diafiltered retentate followed by spray drying adopting standard conditions. The dried retentate is blended with selected additives to produce desired flavour and texture. The dried *rasogolla* mix has about 5 months at 30°C. Production of *rasogolla* mix powder offers following benefits:

- Offers economic use of seasonal and regional milk surpluses.
- Produce sweets of consistent quality at the convenience of users.
- Adaptable to medium and industrial scale dairy processing operations.
- Allows product diversification with manageable investments for improved productivity of the dairy industry.
- The products offer good export potential.

**Rasogolla making from dried mix**

Equal quantities of water is added to the mix powder and kept for about 5 min for rehydration of proteins. Circular balls of about 7g size are rolled out in a manner that no cracks appear on the surface. Balls are cooked in the boiling sugar syrup, (maintained at 60% consistency) for 15 min with plenty of foam around the balls. The cooked balls are transferred into another hot sugar syrup of about 40% consistency. The yield is almost 20% higher than that obtained by traditional method.

**Paneer**

Production of good quality *paneer* using ultrafiltration (UF) has been reported by Sachdeva *et al.* (1993). The process offers advantages like access to mechanisation, uniform quality, improved shelf
life, increased yield and nutritionally better product. The method involves standardisation and heating of milk followed by UF, whereby lactose, water and some minerals are removed. The concentrated mass, which has about 40 percent total solids, is cold acidified to get the desired pH. Till this point, the product is flowable and can be easily dispensed into containers with automatic dispensing machines. The filled containers are then subjected to texturisation by microwave heating. The resulting product has typical characteristics of normal paneer. The yield increases by about 25 percent due to the retention of good quality whey proteins and the slightly increased moisture content.

**Table 1** Compositional comparison between various types of paneers made by the traditional processes and Long life paneer made by the texturizing process.

<table>
<thead>
<tr>
<th>Chemical attribute</th>
<th>Traditional paneers</th>
<th>Concentrated milk paneer</th>
<th>UF paneer</th>
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<tr>
<td></td>
<td>Full fat (5.8%)</td>
<td>Low fat (1.5%)</td>
<td>Skim milk (0.05%)</td>
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<tr>
<td>Fat</td>
<td>23.41</td>
<td>8.60</td>
<td>0.20</td>
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<td></td>
<td>(50.84)</td>
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<td>Protein</td>
<td>18.33</td>
<td>21.56</td>
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<tr>
<td></td>
<td>(39.81)</td>
<td>(56.32)</td>
<td>(72.92)</td>
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<td>*</td>
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<td>(5.22)</td>
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<td>(32.74)</td>
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</tr>
</tbody>
</table>

Figures in parentheses indicate the values on moisture free basis

In another approach, a fully sterilization process has been developed which yields a long shelf life paneer like product (Rao, 1991). Standardised buffalo milk is concentrated partly by vacuum concentration process and partly by employing UF to a level of total solids desired in the final product. After packing in metallised polyester pouches, product is formed by a texturising process at 115°C, which permits concomitant sterilization. The process permits greater product yield due to retention of whey solids, being 35 per cent as compared to 15 per cent obtained by conventional batch process.

**Shrikhand**

The traditional technology allows the whey proteins to drain along with whey during the process of chakka making. These proteins, having high biological value could be recovered in chakka by the application of ultrafiltration to make, so called UF-chakka (Sharma and Reuter, 1992). Chakka and Shrikhand of good sensory quality and meeting PFA standards could be successfully prepared using ultrafiltration technology (Shukla, 2004). In standardized ultrafiltration process, skim milk coagulum
obtained by fermentation of skim milk with yoghurt culture was heated to 60°C for 5 minute with continuous agitation and ultrafiltered up to around 16.60% TS concentration. Whey was then removed from this concentrated coagulum by hanging it in a muslin cloth (eight layered) at room temperature followed by mild pressing to get chakka. Chakka was then kneaded in a planetary mixer with 70% fat cream and sugar to prepare Shrikhand of smooth consistency. UF process resulted in nil fat loss in whey and 20.70% extra recovery of total solids in chakka. The protein content in skim milk chakka through UF process and in shrikhand prepared from it was higher than in traditional process.

Khoa

Khoa from cow milk has been reported to be salty in taste, sticky/pasty in body and texture and slight yellowish in colour. WPC addition has shown to improve the flavour, body and texture, colour and appearance and thereby overall sensory attributes of cow milk khoa. Addition of 5% WPC solids to cow milk improved the flavour, body and texture and colour of khoa prepared (Patel et al., 1993). WPC incorporated cow milk khoa compared well with the traditional buffalo milk khoa.

Though the flavour score for 12% WPC added khoa were higher than other WPC added khoa samples, the improvement was not statistically significant between 8%, 10% and 12% WPC added khoa (Sudhir, 2002). Increased level of WPC increased the grain size of khoa and decreased stickiness/pastiness; however, it also resulted in reduced cohesiveness and increased dryness in the product. Hence, the selection of level of WPC is subject to the requirement of type of khoa intended for further use e.g. Khoa prepared by addition of higher level can be suitable for kalakand like product.

Sudhir (2002) reported that the khoa with added WPC (80) from nanofiltered cow milk scored higher for flavour and overall scores (47 and 91.29, respectively) than khoa from nanofiltered cow milk (45.71 and 90.43, respectively). A definite increase in grain size for WPC added khoa from nanofiltered cow milk was observed. Khoa with added 12% WPC from nanofiltered cow milk scored more in flavour, body and texture (30.86), colour and appearance (13.42) and overall sensory scores than 12% WPC added khoa from cow milk (44.57, 30.36, 12.71 and 87.64, respectively). The scores of khoa from nanofiltered cow milk with added WPC were also comparable to commercial buffalo milk khoa, which scored 47.07, 31.5, 13.79 and 92.35 for flavour, body and texture and colour and appearance, respectively. However, the product obtained from use of nanofiltered cow milk tended to be sticky, which could be because of homogenization effect on cow milk. Nanofiltration of skimmed cow milk followed by standardization to fat : TS ratio of 0.38-0.4 and subsequent khoa making by WPC addition might probably obviate this problem.

Reuter et al. (1990) incorporated 10 and 18% WPC (27.41% TS) solids in buffalo milk for the manufacture of khoa, Greater amount of WPC produced bigger grains in khoa, which is a desirable property for preparing Kalakand - a popular khoa based Indian sweet.

References


Introduction

Fermented milk and milk products have occupied a place of complacency in satisfying the palate and nutritional requirements of human being since the time antiquity. The fermentation is used as a method of value addition and conversion of raw materials by microorganisms and enzymes into various types of products with distinct nutritional and sensory properties. There are a variety of these products throughout India. The popular products in this category are Dahi, Lassi, Butter Milk, Majjige, Kadhi, Raita, Curd Rice, Mishti Dahi, Srikhand etc. The consumption of fermented products is believed to add value to health attributes and also improve the quality of life (Aneja et al 2002). Fermented milk products have been reported to have therapeutic, anticholesterolemic, anticarcinogenic and anticariogenic properties beyond their basic nutritive value. They, contributing to a variety in our gustative desire, have been recognized to provide important nutrients and considered superior over non-fermented dairy products in terms of nutritional attributes as the microflora present produce simple compounds like lactic acid, amino acids and free fatty acids that are easily assimilable.

Dahi

Dahi, Indian curd, is a well known fermented milk product consumed by large sections of the population throughout the country, either as a part of the daily diet or as a refreshing beverage. In India, dahi also known as dadhi is largely made at home using traditional kitchen recipes, involving milk of buffaloes, cows and goats. Generally a mixture of cow and buffalo milk is used. Milk is boiled and cooled, inoculated with dahi starter, usually the left over from the previous day’s stock, and incubated undisturbed at ambient temperature for four to six hours until it acquires a thick consistency. Dahi is generally consumed in its original form as an accompaniment to the meal or it may be converted into raita. Dahi may be consumed as such or as sweet or savoury drink as a dessert containing sugar, spices, fruits, nuts, etc. An extensive all-India survey project on dahi revealed that there are, broadly speaking, two types of dahi prevalent in the country for direct consumption, viz. a sweet/mildly acidic variety with a pleasant flavour, and a sour variety with a sharp, acidic flavour.

The PFA Act defines dahi or curd as a semi-solid product, obtained from pasteurized or boiled milk by souring (natural or otherwise), using a harmless lactic acid or other bacterial cultures. Dahi may contain additional cane sugar. It should have the same minimum percentage of fat and solids-not-fat (SNF) as the milk from which it is prepared. Where dahi or curd, other than skimmed milk dahi, is sold or offered for sale without any indication of the class of milk, the standards prescribed for dahi prepared from buffalo milk shall apply.

The Bureau of Indian Standards (BIS) specifications for fermented milk products are based on the type of culture used in their preparation. Mild dahi is made from mesophilic lactococci. Leuconostocs may be adjunct organisms for added buttery odour and flavour. Sour dahi contains additional cultures belonging to the thermophillic group, which are generally employed in the manufacture of yoghurt. These thermophillic organisms grow rapidly at 37-45°C, producing dahi in less than 4 hours.
Like dahi, yoghurt is a semi-solid fermented product made from a standardized milk mix by the activity of a symbiotic blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* cultures. For brevity we shall term the yoghurt culture organisms as ST and LB and typical dahi organisms as LL. The body and texture of yoghurt depends largely on the composition of milk employed in its manufacture. Although milk of various mammals can be used for making cultured dairy products, their industrialized production is mainly based on milk of cows and buffaloes.

**Classification**

In general, dahi may be classified into two types:

I. For direct consumption.
II. For churning into desi butter (makkhan)

In a country as big as India, the consumers have different taste preferences for traditional products varying from region to region. This made the traditional products available with a varied taste. Dahi is also made in different varieties with region specific tastes. The technological developments have led to the commercialization of this product. Dahi may be classified on the following basis.

According to use
- a) Dahi for direct consumption
- b) Dahi for the production of chakka, shrikhand, lassi and butter milk.
- c) Dahi for the production of desi butter and ghee.

According to consumption
- a) Whole milk dahi
- b) Skim milk dahi
- c) Toned milk dahi
- d) Standard milk dahi
- e) Dahi from special milk

According to flavour
- a) Sweet dahi (acidity not more than 0.7%).
- b) Sour dahi (acidity not less than 0.7%)
- c) Sweetened dahi
- d) Fruit dahi

**Composition**

Dahi made from buffalo milk produces a thick bodied product because of its high SNF content. It is recommended to make dahi/ yoghurt from a mix containing 11-13 percent SNF. The increased protein content in the mix results in a custard like thick consistency following the required fermentation. Higher milk solids also keep the product from wheying off. Dahi prepared from whole milk contains about fat 5-8, protein 3.2 – 3.4 lactose 4.6 – 5.2, Ash 0.70 – 0.72, and titratable acidity 0.60 –0.80 percent.
Method of Manufacture

Traditional Method

In this method dahi is prepared at small scale, either in the consumer’s household or in the confectionary (Halwais) shop. In the household, the milk is boiled, cooled to room temperature, inoculated with 0.5 to 1.0 percent starter (previous day’s dahi or butter milk) and then incubated undisturbed for setting for about overnight. In cold weather, the dahi setting vessel is usually wrapped up with woolen cloth to maintain appropriate temperature. In the confectionary shops, the method employed for preparation of dahi is more or less same except that the milk is concentrated in an open pan before inoculation and usually dahi is set in earthenware.

Fig. 1. Manufacture of Dahi
As stand process on the basis of scientific lines has been developed for dahi making in the organized sector. Fresh, sweet, good quality milk is received, pre-heated and subjected to filtration and clarification. The milk is standardized to 2.5 to 3.0 percent fat and 10 percent solids not fat, pre-heated to 60°C and homogenized single-stage at a pressure of 176-kg/sq cm. The milk is heated to 85 – 90°C for 15-30 minutes, cooled to 22-25°C and inoculated with 1-2 percent of specific dahi starter culture. It is then filled in suitable packaging containers of the appropriate size and incubated at 22-25°C for 16-18 hours. After proper setting of the dahi, the acidity of dahi reaches 0.6 to 0.7 percent and a firm curd is formed. The curd is cooled by circulating chilled water or air around the containers and then transferred to cold room maintained at about 4-5°C. The flow diagram for manufacture of dahi is presented here under (Fig. 1).

Misti Dahi

Misti dahi or misti doi is a popular traditional sweetened fermented milk product. The eastern parts of India, especially in West Bengal, Assam, Bihar and Orissa, the sweetened variety of dahi known as Misti dahi, Lal dahi or Payodhi is quite popular. The product is prepared by the Halwais on a small scale. It is a delicacy of choice during religious festivities and is considered an auspicious item to serve while starting journey or any important work. The product is commonly sold in earthen pots of varying sizes, and served chilled.

Composition of Misti Dahi

Mishti dahi is a fermented milk product, having creamish to light brown colour, firm body, smooth texture, sweet-acidic flavour, and pleasant aroma. As such, there is no prevention of food adulteration (PFA) Act or BIS standards for misti dahi. In the absence of legal standards, misti dahi differs in terms of chemical composition as well as sensory attributes. The quality of misti dahi depends upon the type of milk, level of concentration, and fermentation conditions employed in its manufacture. The typical composition of misti dahi is given in table 1.

Method of Manufacture of Misti Dahi

Traditional method

Traditionally, misti dahi is prepared from cow or mixed milk. The fresh good quality milk is boiled with a required amount of sugar and partially concentrated by simmering over a low fire. This heating is continued for quite some time during which milk develops a distinctive light cream to light brown caramel colour and flavour. The content is then cooled to ambient temperature and cultured with dahi (lactic) culture. It is then filled into earthen pots of consumer size or bulk size vessels and incubated over night. Normally the curd is set within 12-14 hours. After firm setting of curd, it is transferred to a cooler place or stored under refrigeration.

<table>
<thead>
<tr>
<th>Constituent (%)</th>
<th>Low fat</th>
<th>Medium fat</th>
<th>High fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk fat</td>
<td>2-3</td>
<td>4-5</td>
<td>8-9</td>
</tr>
<tr>
<td>Milk SNF</td>
<td>13-14</td>
<td>11-13</td>
<td>10-11</td>
</tr>
<tr>
<td>Sugar</td>
<td>17-19</td>
<td>17-18</td>
<td>17-18</td>
</tr>
<tr>
<td>Total solids</td>
<td>32-35</td>
<td>32-36</td>
<td>35-38</td>
</tr>
</tbody>
</table>
Industrial Production

In the organized sector, mishti dahi is manufactured employing developed technological process. A wide range of milk products for sourcing milk solids is used in the production of mishti dahi. For this purpose, milk solids are used from fresh cow/buffalo milk, cream, skim milk powder (SMP), whole milk powder (WMP), evaporated whole milk, sweetened condensed milk and white butter.

The required ingredients is blended in proper proportion, keeping in view the final compositional standard of the product in terms of fat, SNF, and sugar. There is a need to select fresh and good quality ingredients in relation to microbial and sensory quality. The raw material milk used for mishti dahi preparation should be fresh, free from off-flavours and clot-on-boiling negative.

The most common sweetening agent used in preparation of mishti dahi is cane sugar. Other sweeteners such as corn sugar, corn syrup, and also sugar or maltose can be used as sweetening agent. Some times in preparation of some special varieties of mishti dahi, fresh palm jaggery is used as a sweetener. Commercially cane sugar of high microbiological quality and free from extraneous matter is used as sweetening agent.

Mishti dahi is colored and flavored commonly with caramel. Caramel is prepared from heating sugar and it is available commercially in a viscous form (76% TS). Caramel is soluble in water and having a specific gravity of 1.315 to 1.345. Synthetic flavours like caramel, vanilla, cardamom, rose, pineapple, etc may also be used. Fruits and dry fruit, nuts may also be used for developing a wide variety of mishti dahi.

The most critical and important step in the manufacture of the mishti dahi is the selection of appropriate type of starter culture since it affects the flavour, consistency and acidity development in the presence of sugar and caramel at relatively higher TS levels. As such starter culture is regarded as heart of mishti dahi preparation. Mixed strain culture may be used since it yields a superior product and most reliable under variable processing conditions. The optimum activity of the mishti dahi culture is expected in a narrow temperature range of 40-42°C. Normally a good starter culture with 1.0 percent inoculum develops 0.70 percent acidity within 6-8 hours.

Process

The required quantities of milk, cream, skim milk powder and sugar are blended. Caramel is added normally at the rate of 0.10 to 0.12 per cent. The mix is heated to 80° – 90°C in a vat or a plate heat exchanger. Various time-temperature combinations have been tried but heating the mix to 85°C for 15 minutes resulted in a highly desirable flavour and textural qualities. After heat treatment, the mix is cooled to 40-42°C either employing heat exchanger or by circulating chilled water in the jacket of vat. The starter culture is added to the mix at the rate of 1.0 percent and thoroughly mixed using stirrer. Thereafter, the mix is filled in sanitized cups of required sizes and covered with lids. The cups are properly heat sealed to make them airtight and prevent leakage. These cups are then incubated at 40-42°C for about 6-8 hours till the acidity develops to about 0.70 to 0.80 percent LA. At this acidity the mix will well set and a desired consistency and firmness is attained. After proper setting, these cups are transferred to a cold store of 4-5°C temperature. For long storage, normally the temperature of cold store is maintained at 0°C

Production of Misti Dahi from Buffalo Milk

Fresh buffalo milk is standardized to 3.5% fat and 9.0% SNF, heated to 65°C in a plate heat exchanger and homogenized at a pressure of 56 kg/cm² (one stage). Milk is concentrated at 1.44 fold in a vacuum evaporator. After adding cane sugar, the milk is heated at 85°C for 5 min to generate cooked flavour. The mix is water cooled to 40°C before inoculation with the mixed culture (LF-40). In some
cases, sugar caramel, jaggery and artificial colors are added to impart brown colour. The inoculated mix is aseptically distributed into pre-sterilized polystyrene containers (200 ml) and mechanically transferred to incubation chamber at 40°C. After 7 h of incubation, the product is shifted to cold store maintained at 4°C. During gel formation, milk must remain stationary. In the flow chart (Fig.2), fermentation is designed as a batch process. In all post fermentation activities, gel should be subjected to a minimum amount of external influences.

Whole Buffalo Milk
↓
Standardization (Fat, 3.5%; SNF, 9.0%)
↓
Pre-heating (65°C – 70°C)
↓
Homogenization (56 kg/cm² at 65°C)
↓
Concentration to 1.44 fold
↓
Addition of sugar (approx. 14%)
↓
Heating at 85°C for 10 min
↓
Cooling to 40°C
↓
Continuous pre-fermentation in tanks and main fermentation in packs. (Upto pH 5.2 milk remains Liquid) Inoculation (LF-40 culture @ 1.0%) Aseptic packaging in
↓
Incubation at 40°C for 6-7 h
↓
Storage at 4°C

Fig. 2 Flow diagram for manufacture of Misti Dahi

**Probiotic Dahi**

Probiotic foods are the most important discipline of functional foods, which are defined as foods containing live microorganisms, which actively enhance the health of consumers by improving the balance of microflora in the gut when ingested, live in sufficient numbers. Several studies have related the promising health benefits of consuming cultured and culture containing milks. There have been
long term interests of using cultured milk products with various strains of LAB and other probiotic bacteria to improve the health of humans. The consumption of probiotic products is helpful in maintaining good health, restoring body vigour, and skirmishing intestinal and other diseases. Fuller (1989) listed out the claimed beneficial effects and therapeutic application of probiotic bacteria in humans, which includes: (i) beneficial effects, such as maintenance of normal intestinal realm, augmentation of immune system, reduction of lactose intolerance, reduction of in serum cholesterol levels, anticarcinogenic activity, and improved nutritional value of foods, and (ii) therapeutic applications, such as prevention of urogenital infections, mitigation of constipation, protection against travellers’ diarrhea, prevention of infantile diarrhea, reduction of antibiotic induced diarrhea, prevention of hypercholesterolemia, protection against colon/bladder cancer and prevention of osteoporosis, etc. Probiotic bacteria, thus, offer new dietary alternatives for the management of such conditions through stabilization of intestinal microflora, promotion of colonization resistance, regulation of the immune response and preservation of intestinal integrity.

Recently, at NDRI probiotic dahi has been developed with enhanced health attributes. The probiotic lactobacilli viz. *L. acidophilus* and *L. casei* used to prepare dahi either alone or in combination with mesophilic dahi culture *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis*-60 and mixed dahi culture 167 (BO4). Standardized buffalo milk (fat 4%) as well as milk with different fat % (1 to 3%) is used for preparation of two types of Dahi. Dahi incubation carried out at 37oC for 9-10 hours. After incubation dahi is stored at 4°C (approx.). Dahi exhibited good taste and flavour, also good; texture is firm exhibiting pH 4.27 to 4.47 and titratable acidity ranging from 1.08-1.21. A number of probiotic organisms are 7.1x10^10 approx. Number of probiotic organisms is ranged from 3.8 x10^10 - 4.24x10^10.

**Fruit Dahi**

India is also amongst largest fruit producing countries, with vast horticulture base consisting wide range of fruit varieties. The production of fruits in India is confined to 27.8 MT, which amounts to 8.1% of the total world fruit production. Post harvesting losses in India are also amongst highest in the world at 30-35 % resulting in a great loss to our economy (Indian Economic Review, 2001). The major hurdle in the successes of fruit process industry is the lower demand of processed fruits. The poor purchasing power of Indian consumers and current fruit market structure does not offer a competitive environment to fruit processing industry. Also there is a need to create new avenues for fruit products as our fruit production is increasing rapidly. All these factors demand creation of new avenues for the utilization of fruit products. Keeping in view, the market trend in western dairy market, incorporation of fruits in to fermented milk product would generate a great demand for processed fruits, which might help checking the post harvest losses and the economic loss to the nation and would enhance the profitability of milk and fruit producer as well as processors.

**Manufacture of Fruit Dahi**

The processing parameters for manufacture of fruit dahi have been standardized for the development of good quality fruit dahi using various fruits, such as mango, pineapple and banana (Fig.3). Appropriate starter cultures have been employed to get desired flavour and consistency in the product. The rheological properties of the fruit dahi have been enhanced by incorporation of exopolysaccharide producing cultures and hydrocolloids. The shelf life of the product is about 3 weeks at refrigeration temperature. This newly developed fruit dahi with firm body, smooth texture and with delicate balance of fresh fruity aroma and a typical dahi flavour will have greater aesthetic appeal and will cater to the growing needs of Indian dairy industry. The millions of milk producers and fruit growers will be benefited with this technological development. The technology has tremendous techno-economic feasibility.
Shrikhand

Shrikhand is an indigenous fermented and sweetened milk product having a typical pleasant sweet-sour taste. It is prepared by blending chakka, a semi-solid mass obtained after draining whey from dahi, with sugar, cream and other ingredients like fruit pulp, nut, flavor, spices and color to achieve the finished product of desired composition, consistency and sensory attributes. Shrikhand has a typical semi-solid consistency with a characteristic smoothness, firmness and pliability that makes it suitable for consumption directly after meal or with poori (made of a dough of whole-meal wheat, rolled out and deep-fried) or bread. Although largely produced on small scale adopting age-old traditional methods, shrikhand is now commercially manufactured in organized dairy sector to cater to the growing demand.

The traditional method of making shrikhand involves the preparation of curd or dahi by culturing milk (preferably buffalo milk) with a natural starter (curd of the previous batch). After a firm curd is formed, it is transferred in a muslin cloth and hung for 12–18 h to remove free whey. The chakka obtained is mixed with required amount of sugar, color, flavoring materials and spices and blended to smooth and homogenous consistency (Upadhyay and Dave, 1977).

Aneja et al. (1977) developed an industrial process for the manufacture of shrikhand. Normally skim milk is used for making dahi for the manufacture of shrikhand in this method. By using skim milk,
not only fat losses are eliminated, but also faster moisture expulsion and less moisture retention in the curd are achieved (Patel, 1982). Several attempts have been made to incorporate different additives into shrikhand to address the growing interest in the diversification of food products to attract a wider range of consumers.

**Lassi**

*Lassi* is made by blending *dahi* with water, sugar or salt and spices until frothy. The consistency of *lassi* depends on the ratio of *dahi* to water. Thick *lassi* is made with four parts *dahi* to one part water and/or crushed ice. It can be flavored in various ways with salt, mint, cumin, sugar, fruit or fruit juice and even spicy additions such as ground chilies, fresh ginger or garlic. The ingredients are all placed in a blender and processed until the mixture is light and frothy. While sweetened *lassi* is popular mainly in North India, its salted version is widely relished in the southern parts of the country. Various varieties of salted *lassi* include buttermilk, chhach and mattha. Khurana (2006) developed suitable technologies for the manufacture of extended shelf life mango, banana and pineapple *lassi* along with their low-calorie counterparts using artificial sweeteners.

**Manufacture of Bajra Lassi (Milk-Pearl Millet Based Fermented Beverage)**

A technology has been developed for manufacture of Bajra Lassi (Milk-Pearl Millet Based fermented Beverage) using milk solids and pearl millet which posses health attributes. Development of *Bajra lassi* is based on the selection of milk solids source; selection of level, form & stage of addition of pearl millet 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 is selected as source of milk solids for development of proposed beverage. Pearl millet solids are added to milk in three different forms viz. a) raw flour obtained from milling pearl millet grains, b) slurry obtained by grinding of soaked pearl millet grains and c) flour obtained after grinding of 24 h & 48 h germinated and dried pearl millet grains (malt). These solids are incorporated at two stages i.e. before fermentation and after fermentation. NCDC-167 starter culture is added for fermentation to obtained desirable acidity in the curd. After fermentation salt and spices are added and packaged in suitable size packages (LDPE pouches). The bajra lassi contains TS 9.74%, fat 2.3%, protein 2.5% and ash (including salt and spices) 1.28%. The shelf life of bajra lassi packaged in LDPE films is about seven days at refrigeration storage. The shelf life of bajra lassi is further enhanced from 7 days to 35 and 28 days at refrigeration storage by adding Potassium sorbate and Nisin or MicroGARD, respectively. The technology developed for the production of cereals fortified fermented milk beverage would provide value addition to milk by-product and under utilized cereal, in addition to diversification of activities of dairy processing plants. The salted beverages contains health promoting nutrients derived from fermentation of germinated cereals and milk and thus has great potential to be accepted as substitute of soft drinks (Kanawjia, *et al.*, 2007).

**Conclusion**

Fermented milks have been produced and consumed since ancient times. Dahi has managed its popularity in Indian diet despite changing life styles and food habits with time. It is preferred over milk due to good taste high nutritive and therapeutic value and most importantly, enhanced keeping quality than milk in a tropical climate like ours. It is being consumed as plain, sweetened or salted and spiced. At present several types of fermented milk products such as dahi, misti dahi, fruit dahi, probiotic dahi, lassi, shrikhand, etc. are being produced and marketed. Many people recognized the
dietetic and prophylactic properties of fermented products and their healing effects in certain conditions. As a result, the use of various types of fermented milks has found a wide application.

References


Sensory Evaluation of Milk and Milk Products: Requirements and Technique

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Introduction

Sensory evaluation is the measurement and analysis of a food product’s quality based on information received from the five senses i.e., sight, smell, taste, touch, and hearing. During sensory evaluation of food the signals generated at the nerve endings of the senses are transmitted via the central nervous system to the brain where they are integrated with past experiences, expectations, and other conceptual factors before the option of the response in summarized, interpreted and a decision is made on the attributes of the particular food that matter most (Broune, 2002). Sensory evaluation of milk is of is of special importance because approximately 17 per cent of world total production of milk (703MMT) was consumed as liquid milk in 2009 (IDF, 2010). The liquid milk consumption is even much higher in India. It has been estimated that forty six percent i.e., approximately 56 MMT of India’s total milk production (121.8 MMT in 2010-11) is consumed as liquid milk. Finished milk products can not be better than the ingredients from which they are made. Milk being the major raw material for producing all the dairy products proper sensory evaluation of both raw as well as processed milk is very important and needs special care. There are two broad categories of milk viz., market milk and manufacturing milk. Market milk are meant for direct consumption whereas, manufacturing milks are used for the production of other dairy products. The classes of liquid milks according to the Food Safety and Standards (Food Products Standards and Food Additives) Regulation (FSSR, 2011) of India are presented in Table 1.

<table>
<thead>
<tr>
<th>Classes</th>
<th>Designations</th>
<th>Locality</th>
<th>Milkfat (Minimum)</th>
<th>Milk solids not fat (MSNF) (Minimum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffalo milk</td>
<td>Raw, Pasteurized, flavoured and sterilized</td>
<td>Haryana/ Punjab</td>
<td>6.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Cow milk</td>
<td>Raw, Pasteurized, flavoured and sterilized</td>
<td>Haryana/ Punjab</td>
<td>3.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Mixed milk</td>
<td>Raw, Pasteurized, flavoured and sterilized</td>
<td>All India</td>
<td>4.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Standardized milk</td>
<td>Pasteurized, flavoured and sterilized</td>
<td>All India</td>
<td>4.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Recombe...</td>
<td>Pasteurized, flavoured and sterilized</td>
<td>All India</td>
<td>3.0</td>
<td>8.5</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------------------</td>
<td>-----------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Toned milk</td>
<td>Pasteurized, flavoured and sterilized</td>
<td>All India</td>
<td>3.0</td>
<td>8.5</td>
</tr>
<tr>
<td>Double...</td>
<td>Pasteurized, flavoured and sterilized</td>
<td>All India</td>
<td>1.5</td>
<td>9.0</td>
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<tr>
<td>Skimmed...</td>
<td>Raw, Pasteurized, flavoured and sterilized</td>
<td>All India</td>
<td>Not more than 0.5</td>
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<tr>
<td>Full cream milk</td>
<td>Pasteurized &amp; Sterilized</td>
<td>All India</td>
<td>6.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

Table 1. Classes of liquid milks according to the Food Safety and Standards (Food Products Standards and Food Additives) Regulation (FSSR, 2011) of India.

**Requirements of Sensory Evaluation**

A successful implementation of scientific sensory evaluation programme requires four major components *viz.*, i) Proper sensory evaluation laboratory facilities ii) Selection of sensory panel members/evaluators and iii) Training of the panel members iv) A sensory evaluation score card.

**i) Sensory Evaluation Laboratory:** A design of sensory evaluation laboratory is shown in Fig. 1 (Stone and Sidel, 2004). A sensory evaluation laboratory should include a sample preparation room, a briefing room, testing booths and an office.

**Sample preparation room:** It should be located adjacent to the testing area. Its location shall be such that the evaluators do not have pass through testing area. It should be properly ventilated so that odours emanating from the sample preparations are removed. The main components the preparations room for dairy products are: working space, sink, cooking range, oven, refrigerator, deep freeze, blender, scoops, knives, trier, balance, dishes, spoons, and cleaning and storage facilities. Utensils and cutlery used in sample preparations and presentation shall be of the materials, which do not impart any odour or taste to the product.

**Briefing Room:** Sensory panel members are first assembled here. They are here briefed about the objective of the sensory evaluation programme, score card and it’s use. This room should be adjoining to testing booths and have facilities for comfortable sitting. If enough space is not available for an office for sensory leader or organizer, briefing room should be also serve as office.
Testing Booths: This is the area where panel members carry out actual sensory evaluation of dairy products. Testing area shall be located separately but in the immediate vicinity of the preparation area. This area is normally divided into small booths usually between 5-10 numbers so that the panel members can independently evaluate the product. A layout of the testing booth is presented in Fig. 2 (Pal and Raju, 2009).

Following conditions have to be maintained in testing area for obtaining best results:

- The size of each testing booth shall be sufficiently large to accommodate the sample, utensil, sink, rinsing agents and score card. An area of 0.9 m wide and 0.6 m deep is considered optimum for this purpose. The height of working spaces in the booth should be appropriate to allow comfort to the evaluator.
• The temperature and relative humidity shall be maintained about 20°C and 62% respectively for the comfort of the evaluators. The area should be noise proof and the movements in this area should be restricted.
• The testing area must be kept free from odours. A slight positive pressure may be created in the testing area to prevent the inflow of extraneous odours.
• Adequate lighting is very important in all sensory testing. Lighting particularly in testing booth shall be uniform, shadow free, controllable and of sufficient intensity to permit effective evaluation of the colour and appearance of sample. In this most cases, light having a correlated colour temperature of 6500K (or 110 candle foot light) are desirable. In order to mask difference in colour and other appearance characteristics special lighting devices, such as a dimmer device, colored lamps/ filters or sodium vapors lamps, may be provide.
• A counter with openings, covered by sliding doors, of convenient size should be provided for supplying samples in to the booth from the sample preparation room. A system such as light bulb on the counter side is devised for evaluator to signal to the operator when he is ready for sample.

ii) Selection of Sensory Panel Members

Sensory evaluation work is often time consuming and sometimes cumbersome also. The evaluator should therefore have availability, interest and motivation to participate in training and sensory evaluation programme. He/she should have following qualities:

**Attitude towards foods:** Persons having strong liking or disliking towards a dairy product should not be screened.

**Aptitude and knowledge:** The assessor should have ability to concentrate and must be unbiased. He should have the basic knowledge on the principal sensorial attributes of milk products.

**Health:** The evaluator should be in good health. The sensitivity for evaluator in respect of sense of smell and taste should be normal. He/she should not be suffering from anosmia and ageusia.

**Age:** Evaluators should preferably be in age group of 18-50 years. Person of younger age are unable to properly interpret and communicate the sensory result whereas at older age the memory decrease.

**Adaptation:** Continuous exposure of evaluator to a particular stimulus, particularly at high concentration for long time lead to decrease in his sensitivity (also called as fatigue). It is therefore desirable either to give sufficient to time between the sample or use taste sanitizers, such as brine solution, fruits and mild acids.

**Other requirements:** Use of materials, which are likely to vitiate result, such as smoking chewing pan and taking intoxicants by the evaluator should have lapse of at least an hour before the test. Use of strong odoriferous substances such as perfumes, flavors, hair oil should be avoided by the evaluator as in the testing area.

iii) Training of the panel members

The sensitivity and experience of an evaluator influences the accuracy of results. The evaluator should work like a calibrated instrument and provide reproducible results. The training of the evaluators is therefore, very essential for efficient conduct of sensory evaluation of dairy products. Training develops the ability of the panel members to detect, recognize and describe sensory stimuli related to a particular dairy product. During training the assessors shall be acquainted with the desirable and undesirable attributes of the product, correct terminology, use of score card, scoring technique and
sequence of observations. The sensory evaluation training must start with a large group of people but finally a trained panel comprising of 5-6 members should be retained rejecting who are insensitive or under performer.

iv) Sensory evaluation score card

The evaluation card should be simple, brief, easy to follow and all important sensory attributes are included in it. It should be clearly printed and the matter should be arranged in logical sequence. Terminologies used shall be clear and understandable. Normally 5-8 samples with average intensity of flavor for each sitting are optimum. The amount of each sample 25-50 ml or gm which is sufficient for one full sip or bite. The test should be carried out preferably one hour before or lunch.

Desirable Sensorial Attributes of Milk

Typically flavour of milk should be pleasantly sweet and possess neither a foretaste nor an aftertaste. The natural richness in milk is due to presence of milk fat and the sweetness due to milk sugar (lactose). The colour of cow milk is yellowish and of buffalo milk is white. The colour may vary depending on the extent of mixing the two types milks. There shall be no fat globules/particles on the surface.

Score Card for Milk

A 25 – point score card has been recommended by American Dairy Science Association (ADSA) for the sensory evaluation of milk (Table 2). Full or perfect score is normally awarded when there is no defect in milk, and zero score for an unsalable product.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Perfect Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavour</td>
<td>10</td>
</tr>
<tr>
<td>Sediment</td>
<td>3</td>
</tr>
<tr>
<td>Package</td>
<td>5</td>
</tr>
<tr>
<td>Bacteria</td>
<td>5</td>
</tr>
<tr>
<td>Temperature</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 2. Score card for sensory evaluation of milk

Order of Examination and Scoring Technique of Milk

Containers and closure: The package and its closure in which milk is supplied should be carefully observed. Now-a-days milk invariably packaged in polyethylene sachets. Hence, the evaluator must see that the packaging is no leakage/pilferage from the pouches/containers. The containers should be examined for the extent of fullness (specified amount), cleanliness and printing.
**Sediment:** Milk samples should be observed for the presence of sediments. The kind, amount and size of sediment particles should be carefully observed by visual observation and scored against a chart of mental image. A 3-point scale may be employed occasionally. The presence of any sediment in the processed milk is serious and should receive a zero score. One possible scoring system using a sediment disc is presented in Table 3.

<table>
<thead>
<tr>
<th>Amount of sediment</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sediment</td>
<td>0</td>
</tr>
<tr>
<td>&lt; 0.02 mg/disc</td>
<td>3</td>
</tr>
<tr>
<td>0.02 – 0.025 mg/disc</td>
<td>2</td>
</tr>
<tr>
<td>&gt;0.025 mg/disc</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3. Scoring system of milk using a sediment disc

**Flavour:** For the flavour evaluation, milk should be properly tempered between 13 and 18°C, preferably at 15.5°C. Samples should be served in clean, odourless glass or plastic bottles. For each evaluator, about 50 ml sample should be provided. Immediately after removing the lid the milk sample should be smelled and simultaneously observe for the presence of cream or partially churned fat globules at the top. Then mix the sample properly and take a generous sip, not less than 10 ml of milk, roll it around the mouth and note the flavour and tactual sensation, then expectorate the sample, sometimes, the aftertaste may be enhanced by drawing a breath of fresh air very slowly through the nose. Slow agitation of milk leaves a thin film of milk on the inner surface of the bottle, which tends to evaporate thus readily giving off the odour present.

**Temperature:** Raw milk as well as pasteurized milk should be stored at 7.2°C but lower then 4.4°C is preferred. For pasteurized milk, if the temperature is above 7.2°C, the sample may be scored zero. One point may be given is the temperature is between 4.4-7.2°C. Full two points may be given for a sample at or below 4.4°C.

**Bacterial count:** The maximum permissible bacterial count in pasteurized milk in India is 30000/ml and coliform must be absent in 0.1g. A sample containing a higher count than this limit should get zero score out of five. The practical count, however, cannot be done on every sample of raw milk for judging purpose. Hence, it is recommended to perform the bacterial count test after a certain interval or in case of suspicion. One possible scoring for bacterial count in milk is presented in Table 4.

<table>
<thead>
<tr>
<th>SPC (cfu/ml)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;30000</td>
<td>0</td>
</tr>
<tr>
<td>18000 - 30000</td>
<td>1</td>
</tr>
<tr>
<td>12000 – 18000</td>
<td>2</td>
</tr>
<tr>
<td>6000 – 12000</td>
<td>3</td>
</tr>
<tr>
<td>3000 – 6000</td>
<td>4</td>
</tr>
<tr>
<td>&lt; 3000</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 4. Score card for bacterial count of milk
Defects in milk
Milk is generally considered to have a flavour defect if it manifests an odour, a foretaste, an aftertaste, or does not leave the mouth a clean, sweet, pleasant condition after testing. Off-flavours in milk may be categorized into four major alphabetical groupings (A-B-C-D) (Alvarez, 2009):

Absorbed (barny, cowy, feed, garlic/onion)
Bacterial (acid, bitter, fruity/fermented, malty, rancid, unclean)
Chemical (astringent, cooked, lacks freshness, light oxidized, metal oxidized, rancid)
Delinquency (flat, foreign, salty, unclean)

Absorbed off-flavours
- **Barny**: Transmitted off-flavour due to poor maintenance, ventilation, cleaning of barn. It leaves a persistent, unclean after taste in the mouth.
- **Cowy**: Distinct cow’s breathe like odour & a persistent unpleasant, medicinal, or chemical aftertaste imparted by acetone bodies & alkyl phenols in milk.
- **Feed**: Roughages impart aromatic taints if fed within three hours before milking.
- **Garlic/onion (weedy)**: Recognized by characteristic pungent odour and somewhat persistent aftertaste.

Bacterial off-flavours
- **Acid**: Sour detected by smell & taste due to microbial conversation of lactose into lactic acid.
- **Bitter**: Due to rancidity, specific weeds consumed by cows and certain organisms especially psychrotrophs.
- **Fruity**: Certain microorganisms (e.g., *Pseudomonas fragi*) produce aromatic end products that seriously taint milk. Quickly detected by odour which may resemble of vinegar, pineapple, apple and other fruits.
- **Malty**: Pronounced off-flavour suggestive of malt caused by the growth of *Streptococcus lactic* subsp. *maltigen* bacteria in milk as the result of temp abuse (~18.2°C).
- **Rancid**: Extremely unpleasant flavour due to volatile fatty acids (butyric, caproic, caprylic, lauric and capric acids) formed through enzymatic hydrolysis of fat. Characterized by soapy, bitter, unclean & nauseating after taste.
- **Unclean**: Higher concentration of alkyl phenols leads to unclean flavour.

Chemical off-flavours
- **Astringent**: Tongue and lining of the mouth tend to feel shriveled, almost puckered. Indicates extreme rancidity.
- **Cooked**: Appears when milk is heated 76°C or more, Four types of heat induced flavours i) cooked or sulfurous ii) heated iii) caramelized and iv) scorched. “Moderate heated” flavour is not objectionable but “pronounced” is highly undesirable.
- **Lacks freshness/Stale**: Not pleasantly sweet and refreshing as is typically desired in milk. It can be a fore runner of other oxidized or rancid off-flavours.
- **Metal induced oxidized**: Due to lipid oxidation induced by catalytic action of certain metals. Metallic, oily, cappy, cardboardy, stale, tallowy, paint-like, and fishy are terms used to describe this off-flavour.
• **Light induced oxidized:** Described as burnt protein, burnt feathers, cabbage-like, light-activated, sunlight or sunshine flavour. Light catalyzed lipid oxidation and protein degradation are involved in development of this flavour. The aroma is similar wet card board or wet pap

**Delinquency off-flavours**

• **Flat:** Flatness appears soon after the sample reaches the tongue, partly as a result in marked changes in the mouthfeel. Could not be detected by smell.

• **Foreign (atypical):** caused by improper use of various chemicals e.g., detergents, disinfectants & sanitizers; exposure to fumes from the combustion of diesel, petrol or kerosene; contamination of insecticides, drenching of cows with treatment chemicals; treatment of the udder with medications.

• **Salty:** This-off taste is commonly associated with milk from individual cows that are in the most advanced stages of lactation or with milk from cows that have clinical stages of mastitis. Result in increase of NaCl in the milk & decrease in other mineral samples.

• **Unclean:** This off-flavour may develop by the action of certain psychrophilic bacteria, when storage temperature is too high (>7.2°C)

**General Guide for Scoring and Grading of Milk and Milk Products**

While judging a dairy product, the identification of a defect, if any, is important but equally important is to award correct scores for different attributes so that the difference among the sensory evaluators is minimum. Some of the defects are very serious, for example sour/high acid, rancid, oxidized and cowy flavour in fluid milk whereas others like feed, weedy and cooked flavour are not very objectionable. The scores are thus based on the nature of defect and its intensity. Final grading of samples is done on the basis of total score. A general scoring guide is given below (Table 5) to help evaluators for consistent judging of dairy product

**Table 5. General Scoring Guide for Milk and Milk Products**

<table>
<thead>
<tr>
<th>Quality according to the Grade of Dairy Products</th>
<th>Grade</th>
<th>Defect &amp; Intensity</th>
<th>Approximate Score (% of the Perfect Score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>A</td>
<td>No defect</td>
<td>More than 90%</td>
</tr>
<tr>
<td>Good</td>
<td>B</td>
<td>Flavour: Flat, slight cooked/stale/barny/neutralized/ salty Consistency/Texture: defects of only slight intensity</td>
<td>More than 80% But less than 90%</td>
</tr>
<tr>
<td>Fair</td>
<td>C</td>
<td>Flavour: Definite cooked/neutralized/ feed/ flat. Slight rancid/oxidized/metallic/fishy/ yeasty/mouldy/acidic Consistency/Texture: Any texture defect of definite intensity</td>
<td>More than 60% But less than 80%</td>
</tr>
<tr>
<td>Poor</td>
<td>D</td>
<td>Flavour: Any flavour defect of the higher intensity as given above for grade C Consistency/Texture: Pronounced defect</td>
<td>Less than 59%. The products are generally unacceptable at this score</td>
</tr>
</tbody>
</table>
References


Specialized Dairy Foods for Metabolic Disorders

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Introduction
Metabolism is the sum of the chemical processes and interconversions that take place in the cells and fluids of the body that converts food into energy. This includes the breakdown and buildup of large molecules, their conversion into small molecules, transportation and absorption of nutrients and minerals and the ultimate production of energy from these absorbed molecules. A myriad of metabolic functions are constantly occurring in the body of any living organism, as the cells work together to keep their parent organism healthy. Virtually every chemical step of metabolism is catalyzed by an enzyme. Hence, a major part of a healthy metabolism depends on 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. The disorders usually result from an inability to break down some substance that should be broken down, allowing some intermediate substance that is often toxic to build up, or from an inability to produce some essential substance. Some compounds may build up to toxic levels in the body, because they are not being properly metabolized. In other cases, the host organism may fail 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. Typically, a metabolic disorder is inherited. In some instances, diseases, exposure to toxins, diet, and drug use may cause metabolic disorders. Since the symptoms can be vague, diagnosis is complicated, especially in regions where people do not have access to proper health care.

Metabolic syndrome
“Syndrome” means a combination or group of different symptoms that characterize a specific disease or illness. The metabolic syndrome is connected with a group of symptoms caused by the malfunctioning metabolic processes and linked to the existence to specific diseases. The World Health Organization (WHO) criteria of metabolic syndrome are:

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

**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 1 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.

**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:

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, yoghurt, 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.

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 to make in hypolipidaemic.
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.

**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 as well as 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.

**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. The presence of MA is basically 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 diet 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 weight 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 micro-filtration for selective removal of casein as well as whey proteins could be used for developing lowering protein in dairy products.

**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 them are lactose-malabsorbers.

**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 desorption 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.

**Conclusion**

On account of increased consumption of energy rich foods having high amount of sugar and fat content coupled with sedentary lifestyle, has opened gateway for non communicable disorders like obesity, diabetics and cardiovascular diseases to creep in to the human life which are also known as metabolic disorders. Developing low fat dairy products, fortifying them with functional ingredients, and using modern technological interventions it is possible to develop dairy products that can help to combat and cure metabolic disorders.
Bibliography


Table 1. Glycemic Index of common foods

<table>
<thead>
<tr>
<th>Dairy Products</th>
<th>Snacks</th>
<th>Sugars</th>
<th>Vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk (whole) 22</td>
<td>Cashews 22</td>
<td>Fructose 23</td>
<td>Beets 69</td>
</tr>
<tr>
<td>Milk (skimmed) 32</td>
<td>Peanuts 14</td>
<td>Glucose 100</td>
<td>Cabbage 10</td>
</tr>
<tr>
<td>Milk (chocolate flavored) 34</td>
<td>Popcorn 55</td>
<td>Honey 58</td>
<td>Carrots 49</td>
</tr>
<tr>
<td>Ice Cream (full-fat) 61</td>
<td>Potato Chips 55</td>
<td>Lactose 46</td>
<td>Corn 55</td>
</tr>
<tr>
<td>Ice cream (low-fat) 50</td>
<td>Noodles (instant) 46</td>
<td>Maltose 105</td>
<td>Green Peas 48</td>
</tr>
<tr>
<td>Yogurt (low-fat) 33</td>
<td>Walnuts 15</td>
<td>Sucrose 65</td>
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<tr>
<td>Cheese 0</td>
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<td>Potato (baked) 93</td>
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Development of Probiotic Traditional Dairy Products

Latha Sabikhi
Senior Scientist
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Introduction

The human gastrointestinal tract (GIT) is a complex ecosystem, which contains more than 400 species of bacteria. Some are beneficial, whereas others are potentially dangerous. The mechanisms regulating the number, type and metabolic activity of this diverse array of microbes are quite complex. However, in general, beneficial bacteria can help prevent the pathogenic activity of some invading microbes. Their number diminishes with age, the use of antibiotics, stress, alcohol consumption and other factors. Probiotics are defined as living bacteria that, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001). To boost the beneficial attributes of the normal flora, it is theorized that consumers can reintroduce large numbers of these bacteria (probiotics) to their GIT through oral consumption, thus strengthening the body’s defense against potentially harmful bacteria. Metabolic activity by these probiotic cultures, as well as their competitiveness, can positively influence the ratio of desirable bacteria to undesirable bacteria. Table 1 lists some organisms that have proven probiotic attributes.

Cultured dairy product manufacturers have believed for more than a century that milk is the ideal vehicle for delivering probiotics. There are significant nutritional benefits with consuming a whole food as opposed to taking a medicine. These benefits include a food’s inherent nutrients, such as vitamin, mineral, amino acid, carbohydrate and fatty acids. Examples of probiotic-containing foods can be found in several western traditional cultured dairy products like yogurt and fermented milk. These products are recognized for their health attributes, which range from catering to lactase-deficient individuals to enhance lactose digestion through preventing and treating diarrhea to exhibiting immune-enhancing properties. Table 2 lists some of the reported benefits of probiotic organisms. Responsible development of effective probiotic dairy foods requires conclusive scientific evidence of the claimed probiotic effects on human health. Identification and characterization of probiotic cultures, confirmation of their viability through a dairy product’s shelf life and digestion and ensuring safety of the organisms are the inevitable steps of these scientific investigations.

Probiotics – the Indian scenario

Within the functional foods regime, probiotics is a rapidly expanding, active arena. Being the largest producer of milk and having world’s highest cattle population, India can play a key role in the probiotic revolution. Indian probiotic industry is in its infancy stage and presently accounts for only a small fraction i.e. less than 1% of the total world market turnover in the probiotic industry. But probiotic industry is evolving at a steady pace and is set for tremendous growth in near future. India is emerging as a major probiotic market of the future with annual projected growth rate of 22.6% and turnover of $8 million by 2015. The major players in Indian probiotic industry are Amul, Mother Dairy, Yakult Danone and Nestle along with several minor participants operating in different regions in their own capacities. Probiotics in India generally comes in two forms, milk and fermented milk products with the former occupying 62% of the market share and the latter having 38% market.

Amul was the first to make a dent at national level with its probiotic ice creams ProLife in February, 2007, followed by probiotic lassi. Probiotic products contribute to 10% of Amul’s ice-cream sales and
25% of its dahi sales. The recommended daily dosage of Amul probiotic dahi is 70 to 100 gm. Mother Dairy has the largest milk (liquid/unprocessed) plants in Asia selling more than 25 lakh liters of milk per day. The company’s probiotic products are b-Activ Probiotic Dahi, b-Activ Probiotic Lassi, b-Activ Curd and Nutrifit (Strawberry and Mango) are the company’s probiotic products. Probiotic products contribute to 15% of the turnover of their fresh dairy products. Nestle Nesvita was India’s first dahi with probiotics for healthy digestion. Nestle ActiPlus contains a unique strain of probiotic Lactobacillus acidophilus. The firm claims that the unique action in the intestine delivers many positive benefits which lead to a healthy digestive system and that the product contains $10^7$ probiotic organism per gram. Yakult Danone India Pvt. Ltd. is a 50:50 joint venture between Japan’s Yakult Honsha and the French Danone Group and is offering Yakult, a probiotic drink made from fermented milk, L. shirota and some sugar. The entry of Yakult is expected to increase the visibility and growth of probiotic category in India. Major players in the probiotics drug market in India include companies like Ranbaxy (Binifit), Dr. Reddy's Laboratories, which has four probiotic brands, Zydus Cadila, Unichem, JB Chem, and Glaxo SmithKline. While probiotics in the form of drugs are widely accepted, probiotic foods are still viewed with scepticism. Acceptance is growing slowly, but it will take a long time while before changing the mindset of Indian consumers.

Research on probiotics in traditional Indian dairy products

Research initiative to incorporate probiotics in Indian dairy products is a fairly recent phenomenon. Much of the work is centred around fermented products such as dahi and lassi. Pawan and Bhatia (2007) conducted clinical studies by feeding human volunteers commercial samples of probiotic dahi and lassi containing Lactobacillus and Streptococcus species. The results showed significantly enhanced immune response by probiotic consumption. Non-significant reduction in total and HDL cholesterol level in the subjects and decrease in systolic blood pressure in hypertensive patients was also observed. It is suggested that probiotic diet therapy can be a safe additive or alternative to existing drug therapy. Sinha and her co-workers developed probiotic dahi containing probiotic L. acidophilus and L. casei and investigated their probiotic potential. They investigated the antidiabetic (Yadav et al., 2007, 2008a,b), antibacterial (Jain et al., 2008), immunomodulating (Jain et al. 2009) and antiallergic (Jain et al., 2010) potential of the product. A fermented beverage with high probiotic counts ($10^{11}-10^{12}$ of L. acidophilus NCDC 13) with antidiaarrhoal (Ganguly and Sabikhi, 2012) and potential anticarcinogenic (Ganguly and Sabikhi, 2013) properties was prepared from a combination of whey, skim milk, pearl millet and barley malt. Fermentation by the probiotic organism helped to reduce the antinutrient content in the cereals (Ganguly et al., 2011).

Scientists at NDRI have been working in the field of probiotics and have a repository of 120 types of bacteria (Sachan, 2012). Two strains Lactobacillus plantarum-91 (Lp-91) and Lactobacillus fermentum-1 (Lf-1) have shown promising traits. Lp-91 has been shown to lower the cholesterol in animals by 21%, while Lf-1 has been found to be effective against colitis in mice. The workers plan to screen all bacterial samples to see which ones can increase the feeling of satiety (make a person feel full), which may help overcome India’s increasing obesity epidemic. Scientists at Anand Agricultural University have been working on L. helveticus and L. rhamnosus for the past 25 years. They have standardised several types of dahi (curd), lassi (sweet butter milk) and butter milk which contain these organisms.

There exists a debate on the origin of the probiotic organism. While one group of scientists says that a probiotic of Indian origin only will work in Indian products, another group refutes this argument. The former is based on the fact that gut flora of a specific community is well adapted to the environment of their intestine, while foreign probiotic bacteria face tough competition from these microbes as they have originated from the gut of a population having different food habits. Local factors, like the
environment in which a person lives, influence the gut environment. So Indian strains may have an advantage in that respect and can become part of the Indian gut very easily since they have been isolated from a similar gut environment and are well adapted to survive therein. The other group feels that there is no scientific proof of this assumption. They argue that probiotics colonise the gut only transiently and are supposed to get washed out anyway, which is why probiotic products should be a part of the daily diet.

Conclusion

With the current focus on disease prevention and the quest for optimal health, the use of probiotic market potential is enormous. Already, probiotic food products are popular in Japan, USA, Europe and Australia. The role of probiotics in improving intestinal microbial balance, prevention of cancer, lowering of high cholesterol levels and improving bioavailability of dietary calcium, are the most promising areas for further research. There is also a greater need for selection and developing of starter cultures, especially the mixed cultures, for preparing new probiotic products.

Selected reading


**Table 1—Bacterial cultures with proven probiotic attributes**

<table>
<thead>
<tr>
<th>Species</th>
<th>Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bifidobacterium adolescentis</em></td>
<td>-</td>
</tr>
<tr>
<td><em>B. bifidus</em></td>
<td>Bb-11</td>
</tr>
<tr>
<td><em>B. breve</em></td>
<td>Yakult</td>
</tr>
<tr>
<td><em>B. essensis</em></td>
<td>Danone, (Bio Activia)</td>
</tr>
<tr>
<td><em>B. infantis</em></td>
<td>Shirota, Immunitass, 744, 01</td>
</tr>
<tr>
<td><em>B. lactis</em></td>
<td>Bb-02</td>
</tr>
<tr>
<td><em>B. laterosporus</em></td>
<td>CRL 431</td>
</tr>
<tr>
<td><em>B. longum</em></td>
<td>B536, SBT-2928</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em></td>
<td>La2, La5 (also known as La1), Johnsonii (La1; also known as Lj1), NCFM, DDS-1, SBT-2062</td>
</tr>
<tr>
<td><em>L. bulgaricus</em></td>
<td>Lb12</td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td>Shirota, Immunitass, 744, 01</td>
</tr>
<tr>
<td>species</td>
<td>strain/strain characteristics</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td><em>L. fermentum</em></td>
<td>RC-14</td>
</tr>
<tr>
<td><em>L. helveticus</em></td>
<td>B02</td>
</tr>
<tr>
<td><em>L. lactis</em></td>
<td>La1</td>
</tr>
<tr>
<td><em>L. paracasei</em></td>
<td>CRL 431</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>299v, Lp01</td>
</tr>
<tr>
<td><em>L. reuteri</em></td>
<td>SD2112 (also known as MM2)</td>
</tr>
<tr>
<td><em>L. rhamnosus</em></td>
<td>GG, GR-1, 271, LB21</td>
</tr>
</tbody>
</table>

Source: Krishnakumar and Gordon (2001)

Table 2. Selected health-related attributes of probiotics used in fermented milks

<table>
<thead>
<tr>
<th>Effects</th>
<th>Health benefits claimed/ published</th>
<th>Health benefits established*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiological effects</td>
<td>• Antagonistic effect against pathogens</td>
<td>•</td>
</tr>
<tr>
<td></td>
<td>• Bile, pH resistant strains, enzymatic activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Production of bacteriocins</td>
<td></td>
</tr>
<tr>
<td>Actions on the digestive tract</td>
<td>• Adherence to human intestinal cell line cultures (in vitro)</td>
<td>Enhancement of lactose</td>
</tr>
<tr>
<td></td>
<td>• Enhancement of lactose digestion in lactase-deficient people</td>
<td>digestion in lactase-deficient</td>
</tr>
<tr>
<td></td>
<td>• Prevention of intestinal disturbances</td>
<td>people</td>
</tr>
<tr>
<td></td>
<td>• Regulation of intestinal motility</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Stabilization of Crohn’s disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Stimulation of intestinal immunity in animal models</td>
<td></td>
</tr>
<tr>
<td>Alterations of the intestinal</td>
<td>• Balance of intestinal bacteria</td>
<td>• Alleviation of lactose</td>
</tr>
<tr>
<td>microflora</td>
<td>• Colonization of intestinal tract</td>
<td>intolerance symptoms</td>
</tr>
<tr>
<td></td>
<td>• Decrease of fecal enzyme activities thought to play a role</td>
<td>• Decrease of certain fecal</td>
</tr>
<tr>
<td></td>
<td>in conversion of pro-carcinogens to carcinogens</td>
<td>enzyme activities</td>
</tr>
<tr>
<td></td>
<td>• Decrease of fecal mutagenicity</td>
<td>• Increase in the number of</td>
</tr>
<tr>
<td></td>
<td>• Increase in fecal bifidobacteria</td>
<td>fecal bifidobacteria</td>
</tr>
<tr>
<td>Actions on diarrhea</td>
<td>• Prevention and/or treatment of acute diarrhea</td>
<td>• Decreased duration of</td>
</tr>
<tr>
<td></td>
<td>• Prevention and/or treatment of rotavirus diarrhea</td>
<td>rotavirus diarrhea</td>
</tr>
<tr>
<td></td>
<td>• Prevention of antibiotic-associated diarrhea</td>
<td>• Treatment of persistent</td>
</tr>
<tr>
<td></td>
<td>• Treatment of persistent diarrhea</td>
<td>diarrhea in children</td>
</tr>
<tr>
<td></td>
<td>• Treatment of relapsing <em>Clostridium difficile</em> diarrheaa</td>
<td></td>
</tr>
</tbody>
</table>
| Systemic effects | • Alleviation of clinical symptoms in children with atopic dermatitis  
• Antagonism against carcinogenic bacteria  
• Beneficial effects in superficial bladder and colon cancer  
• Immune enhancer  
• Reduction in the risk of various cancers  
• Reduction of hypertension in animal models and in humans  
• Reduction of serum cholesterol  
• Stimulation of gamma-interferon production by human blood mononuclear cells in culture  
• Stimulation of phagocytic activity |

* More than one publication with no conflicting data in humans

Emerging Packaging Systems and their Potential Applications for Traditional Dairy Products

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Introduction

At present, India is world’s largest milk producer with an annual production of about 134 million tones (Bhasin, 2013). Of the milk produced in India, about 54% is produced by buffaloes, 24% by crossbreds and 22% by indigenous cattle (Patel, 2013). Pattern of milk consumption in India indicates that 50 – 55% of the total milk produced is utilized for the manufacture of a wide range of traditional milk products viz., fat rich (ghee), heat desiccated (khoa and khoa based sweets, rabri, basundi, brown peda or lal peda, etc.), acid coagulated (paneer, chhana and chhana based sweets), fermented (dahi, misti dahi, chakka and shrikhand), cereal based (rice kheer, chhana kheer, payasam, etc,) and frozen (kulfi) products (Londhe et al., 2006). The production and marketing of these products has largely been in the hands of small and petty traders and halwais. They generally use the batch methods for product preparation without placing any reliance on raw milk quality, hygienic considerations and packaging. A number of surveys conducted on the market quality of indigenous milk products have revealed alarmingly high incidence of microbial contamination, besides large variations in chemical composition, flavour and texture. Though lot of research and development (R&D) efforts have been made for mechanization and upgradation of these methods, due attention has not been paid for designing and developing new packaging materials and systems. In recent years, milk based sweets have been gaining significant importance in Indian dairy industry and also the popularity for the traditional products like ghee, paneer, rasogolla and burfi have increased in western countries and a lot of export potential exists for these products.

In the present WTO regime, as our food supply stems from diverse sources from across the world, there is a need for safe and secure management system(s) along the entire food supply chain. This is especially true and becomes dire necessity for perishable food products. Packaging has a significant role in the food supply chain and is an integral part both of the food processes and the whole food supply chain (Rao and Raju, 2010). Food packaging performs a number of disparate tasks: it protects the food from contamination and spoilage; it makes it easier to transport and store foods; and it makes advertising meaningful and large-scale distribution and mass merchandising possible. However, due to increased demands from consumers in terms of product safety, shelf-life extension, cost efficiency, environmental issues and convenience, food packaging no longer has just a passive role in protecting and marketing the product. In order to improve the performance of food packaging in meeting varied demands of stake holders in the entire supply chain, many new “extra” functions have been introduced in packaging technologies to make it active. In this direction new and innovative packaging technologies such as modified and controlled atmosphere packaging, active and intelligent packaging, antimicrobial packaging and nanopackaging technologies are being developed, tested and optimized around the world (Han, 2005; Raju and Singh, 2013; Ganguly et al., 2013).

In view of the social, religious, cultural, medicinal and economic significance of traditional Indian dairy products, there is an obvious need to extend the shelf life of these products by adopting not only good manufacturing practices but also by packaging them in cheap, convenient and attractive
Modified Atmosphere Packaging

The intrinsic properties of individual dairy and food products are responsible for quality changes and also it is evident that their shelf life is limited in the presence of normal air. There are two principal factors that are responsible for the deterioration of dairy products in presence of normal air. The first being the chemical effect of atmospheric oxygen and second the growth of aerobic spoilage microorganisms. These factors bring about changes in odor, flavor, color, and texture leading to an overall deterioration in quality either individually or in association with one another. The modification of the atmosphere within the package by reducing the oxygen content while increasing the levels of CO$_2$ and/or N$_2$ has been shown to significantly extend the shelf life of perishable foods at chill temperatures. The principle of modified atmosphere packaging (MAP) involves the removal of air from the pack and its replacement with a single gas or mixture of gases by either passive or active methods. The three major gases used in the MAP of foods are O$_2$, N$_2$, and CO$_2$ (Raju et al., 2007).

Several studies have been carried out to enhance the shelf-life of popular khoa-based confections such as brown peda, lal peda and similar variants by applying MAP. In a study conducted by Alok et al. (2013), lal peda samples were packed in a 5-layered packaging bags filled with 3 different gaseous compositions (Air, 70 % N$_2$: 30 % CO$_2$ and 98 % N$_2$) and stored at 10 °C. It was reported that the samples packed with air showed significantly higher chemical deterioration and microbial spoilage as compared to the other two combinations. Further, the study revealed that the shelf life of MAP lal peda containing mixture of gases (N$_2$: CO$_2$ = 70:30) was higher (60 days) compared to nitrogen rich atmosphere (98 %). In another study, Londhe et al. (2012) successfully attempted to improve the shelf-life of brown peda by studying the effect of different packaging techniques on the sensory, physico-chemical, textural, biochemical and microbiological quality during storage for 40 days at 30±1°C. It was reported that brown peda was packaged in a 5-layer nylon-based material (LLD/BA/Nylon-6/BA/LDPE) and flushed with nitrogen and carbon dioxide gases both at 1 bar pressure for 3 s and also vacuum packaged (37.33 KPa for 7.5 s). Brown peda packaged in cardboard boxes lined with butter paper was treated as control in the study. It was reported that the rate of loss of most quality attributes was rapid in control and modified atmosphere packaged samples compared to vacuum packaged samples. Based on the results obtained in the study it was concluded that brown peda could be best preserved up to 40 days in vacuum packaging without appreciable quality loss.

Several attempts were also made for enhancing the shelf life of heat-and-acid coagulated products such as paneer and chhana-based confections. Rai et al. (2008) studied the effect of MAP (vacuum; 100% CO$_2$; 100% N$_2$) on the chemical quality of paneer packaged in high barrier bags and reported that maximum chemical (titratable acidity, FFA and tyrosine) changes were observed in in samples packaged under air while minimum changes were observed in 100% CO$_2$ packaged samples. Srivastava and Goyal (2009) studied the electrophoretic pattern of proteins using SDS-PAGE and reported that maximum proteolysis had been in case of paneer samples (3°C) packaged under air followed by vacuum, 100% N$_2$, N$_2$:CO$_2$ = 50:50 and 100% CO$_2$. Recently, Thippeswamy et al. (2011) reported that combination of hurdle technology (water activity, pH, potassium sorbate) and MAP (N$_2$:CO$_2$ = 50:50) extended the shelf life of cow milk paneer from 1 to 12 days at room temperature (30°C) and 6 to 20 days at refrigeration temperatures (7°C). Paneer tikka, an exotic kebab of Indian cottage cheese is extensively used as fast-food during get-togethers, marriage parties, birthday parties and also at quick service restaurants. The normal shelf life of paneer tikka is low due to microbial and
physico-chemical changes during storage. Successful attempts were made by Ahuja and Goyal (2013) to control the chemical changes during storage (3°C) adopting vacuum packaging of paneer tikka and reported that the shelf life of vacuum packed paneer tikka increased up to 40 days (about 200% increase), in high barrier packaging materials compared to polyethylene films.

Researchers at the Southern Regional Station of NDRI have been continuously working to extend the shelf life of region specific traditional dairy products such as chhana podo and kunda. Recently, chhana podo and kunda were packed in high barrier EVOH pouches and PET laminates under different atmospheres (air, vacuum, 100% CO₂, 100% N₂). It was reported that vacuum packaging did retard microbial growth, but adversely affected the textural and sensory attributes of both products (NDRI, 2010).

Active and Intelligent Packaging

Besides providing a protective atmosphere, packaging material itself may play an active role in enhancing the shelf life of product by nullifying the rate of deteriorative reactions, by arresting the growth of spoilage/pathogenic microorganisms. This has led to the concept of active packaging, the idea which was first floated by Labuza (1987). Active packaging technologies involve interactions between the food, the packaging material, and the internal gaseous atmosphere and play a dynamic role in food preservation. Active packaging senses environmental changes and respond by changing its properties. In other terms, active packaging is a group of technologies in which the package is actively involved with the food products or interacts with internal atmosphere to extend shelf life, while maintaining quality and safety. It is achieved by the use of absorbers, emitters, scavengers, scrubbers, and desiccants that, when added to a package, alter the package structure, function, or properties (Brandenburg, 2009). Diverse functions that the active substances perform include oxygen scavenging, anti-microbial activity, moisture control, ethylene removal, antioxidative reactions, etc.

The active component may be part of the packaging material or may be an inert or attachment to the inside of the pack. Intelligent packaging is designed to monitor and communicate information regarding the present properties of the food, or records aspects of its history about food quality to the consumer (Brody, et al., 2008). It is also more loosely called as smart packaging. It involves devices attached as labels, incorporated into, or printed onto a food packaging material that offer enhanced possibilities to monitor product quality, trace the critical points, and give more detailed information throughout the supply chain.

Although this technique has been applied for dairy products in the Western world, it has not yet received importance in India mainly due to non-availability of active agents locally. However, successful attempts were made at NDRI to extend the shelf life of chhana podo and kunda by applying active packaging technique. It was reported that oxygen scavenger (Ageless®) coupled with high-barrier EVOH and PET laminates extended the shelf-life of chhana podo to more than 45 days and that of kunda to more than 80 days at 30°C without any appreciable loss of sensory and textural characteristics (NDRI, 2010). Recently, Chaturvedi (2011) applied oxygen scavengers (Ageless®) to extend the shelf life of khoa-jalebi, another region specific dairy product and reported that the shelf life of the product increased from 10 days to 42 days when stored at 30°C and 65% RH.

Antimicrobial Packaging Systems

Antimicrobial packaging is a type of active packaging. The antimicrobial agents may be present in the packed food or packaging material itself to reduce, inhibit or retard the growth of microorganisms. Such packaging materials could play an important role in extending shelf-life of foods and reduce the risk from pathogens. Antimicrobial food packaging can take several forms such as (a) addition of sachets / pads containing volatile antimicrobial agents into packages, (b) incorporation of volatile and
non-volatile antimicrobial agents directly into polymers, (c) coating or adsorbing antimicrobials onto polymer surfaces, (d) immobilization of antimicrobials to polymers by ion or covalent linkages, and (e) use of polymers that are inherently antimicrobial (Raju et al., 2013). These systems have not yet thoroughly studied for Indian dairy products although few attempts were made in the recent past to enhance the shelf life of khoa and paneer at NDRI.

**Edible Films and Coatings**

The concept of using an edible film or coating to extend the shelf life of fresh foods and protect them from harmful environment is not a novel one. The idea derives from the natural protective coating such as the skin of some fruits and vegetables. Edible packaging consists of edible films, sheets, coating and pouches. Edible films and sheets are stand-alone structures that are preformed separately from the food and then placed on or between food components or sealed into edible pouches, whereas edible coatings are then placed on or between food components or sealed into edible pouches. Whereas edible coatings are thin layers of edible materials formed directly onto the surface of food (Janjarasskul and Krochta, 2010). The edible films comprise of thickness of less than 254 µm, whereas edible sheets include up to thickness of 254 µm (Ganguly et al., 2013). The edible packaging materials offer multifunction, like offer a selective barrier to retard the migration of moisture, gas transport, oil and fat migration and solute transport; improve the mechanical handling properties of foods; improve the mechanical integrity or handling characteristics of the food; retain volatile flavour compounds and carry food additives such as antioxidants and antimicrobials (Falguera et al., 2011).

Although edible coatings look simple, its application for traditional dairy products is rather tricky as one has to maintain the unique sensory attributes of these products. In this direction some attempts are being made to enhance the shelf life of traditional dairy products by developing edible antimicrobial films (Priyanka et al., 2013). Also, attempts have been made to improve the shelf-life of paneer using WPC-based edible coatings in combination with vacuum packaging using low density polyethylene (LDPE). The coated paneer were packed with vacuum in LDPE and stored at different temperatures. It was reported that the edible coated and vacuum packed paneer samples showed significant effect on the titratable acidity, pH, total viable counts and yeast and mould counts and had a shelf-life of 56 days at 5°C (Archana et al., 2012).

**Biodegradable Packaging**

The rapid industrialization, population explosion and changing life-styles are leading to increased demand for processed and packaged foods. Although there is a wide variation in the level of processing of different food materials, the size of semi-processed and ready-to-eat packaged food industry is over Rs. 4000 crores and is growing at over 20%. The food industry has been largely depending on the petroleum-based plastics for packaging materials. The major reasons for the popularity of plastic packaging materials are their excellent functionality, ease of processing, light weight and low cost. However, in spite of their versatility, a limiting property of most plastics in food packaging is their poor barrier to gases and vapors, including oxygen, carbon dioxide and organic vapors. Further, they are considered menace to environment once they reach municipal solid waste (MSW) and/or landfills or as they not biodegradable (Raju and Singh, 2013). Food packaging is the largest user of plastics (~40%). In India, as per the Central Pollution Control Board, approximately 15,300 tonnes of plastic waste is generated per day (The Hindu, 2011). The volume of plastics discarded annually creates a substantial waste which is causing a great threat to environment. Consequently, the approach of making packaging materials from biodegradable materials that can be disposed of through composting or recycling got momentum. As a result a number of biodegradable materials such as naturally occurring polymeric materials, polymers made by polymerization of
organic molecules and biodegradable polymers from petrochemicals have been investigated for use as alternative to plastics. Biopolymers from agricultural food stocks, food processing waste and other resources have the ability upon blending and/or processing to result in biopolymeric packaging material called as biodegradable polymers or bioplastics (Davis and Song, 2006). Although polylactic acid (PLA) based biodegradable packaging materials are already on shelves in many of the developed nations, in India studies have to be carried out to check the suitability of such materials for Indian dairy products.

**Nanocomposite Packaging Materials**

Polymer nanocomposites are created by dispersing an inert, nanoscale filler throughout a polymeric matrix in which the filler has at least one dimension smaller than 100 nm. Filler materials could be either flakes, fibers, whiskers or nanoparticles. The mechanical, thermal and barrier of nanocomposites are often remarkably different from those of non-reinforced synthetic or biopolymer-based materials. Addition of relatively low levels of nanoparticles (less than 5%) have been shown to substantially improve the properties of finished plastic, increasing the deformability and strength, and reducing the electrical conductivity and gas permeability. Filler materials which have been used widely used by researchers include clay and silicate nanoplatelets, silica (SiO$_2$) nanoparticles, carbon nanotubes, graphene, starch nanocrystals, cellulose-based nanofibers or nanowhiskers, chitin or chitosan nanoparticles, silver nanoparticles (AgNO$_3$), titanium nanoparticles (TiO$_2$), magnesium nanoparticles (MgO), copper nanoparticles (CuO), zinc (ZnO), etc (Raju and Singh, 2013). It has been said by many experts that nanocomposite packaging materials is a silent revolution in the food packaging industry. Although attempts have been made by several workers at CFTRI, Mysore, IIT, Kharagpur, MGU, Kottayam and elsewhere in India to develop and characterize the polymer nanocomposite materials their use in food packaging is rather just a beginning.

**Retort Processing**

Retort processed foods offer long-life and convenient products for which packaging materials play an important role. Successful attempts were made to enhance the shelf-life of ready-to-eat *kheer* by developing an in-pouch (*polyester/aluminium/polypropylene laminate*) retort processing method by Alok *et al.* (2011). It was reported that the product was found to be acceptable for a period of 150 days at 37 °C. *Kunda*, a heat desiccated dairy product was packaged in pouches and retort processed in order to enhance the shelf-life. The packaged and retort processed *kunda* was stored at 37°C and 55°C and the changes in rheological, microbial and sensory quality were monitored at regular intervals by Navajeevan and Rao (2008). It was reported that during storage the processed *kunda* showed a gradual increase in its rheological (firmness, consistency and adhesiveness) properties indicating that retort processed *kunda* became firmer and more adhesive during storage. It was concluded that the spoilage of retort processed *kunda* was more of physical in nature than a microbial one. *Chhana roll*, another indigenous milk-based sweet of the Indian subcontinent is sold at cottage scale without any packaging. In order to enhance its shelf-life, attempts were made to adapt retort processing by processing for 15 min in combination with use of nisin (118.73 IU/g) and found that such retort processed product had a shelf-life of 90 days at 37°C (Jat *et al.*, 2013). Recently, Anuj *et al.* (2013) reported that the shelf life of retort processed dietetic *chhana kheer* was stable up to 90 days at 37 °C.

**Conclusion**

The popularity and commercial significance of traditional milk products is tremendously increasing among the Indian masses as well as among the Indian diaspora living worldwide. These products are also gaining significant importance in terms of export potential. To attract people globally, the dairy
industry must comply with international standards in terms of packaging requirements by carefully selecting the packaging material specific to a product that not only provides convenience in transportation but also improves the shelf life. With innovations that have taken place in packaging materials and systems, the Indian dairy industry must gear-up to adopt the proven systems for improved production and possible extension of shelf life and the R&D efforts need to be made to apply emerging packaging techniques such as biodegradable and nanocomposite packaging.

References


Technology of Low-Calorie Traditional Milk Products

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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. It is predicted that the Indian diabetic population would rise to more than 80.9 million by the year 2030. An Indian National Urban Diabetes Survey reported the average diabetes prevalence rate as 12.1%. 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, the key lifestyle interventions being physical activity and a nutritional plan with reduced caloric intake. In India, non-communicable diseases caused 5.10 million deaths in the year 2002, of which cardiovascular diseases were responsible for 2.78 million deaths. However, there are large disparities in cardiovascular disease mortality in different Indian states. 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 cardiovascular disease mortality (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 fat replacers and 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. 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 and fat replacers have been developed and some are already being witnessed in the super market shelves. Some of the R&D efforts in this area are discussed here.

Low-calorie traditional dairy products

Khoa-based products

Burfi

Burfi, the most popular khoa based confection among Indian traditional dairy products, has its own distinguished niche in Indian diets during festive season as well as day-to-day life. It contains high amounts of fat (20%) and sugar (30%). Successful attempts were made by Prabha and Pal (2006) in developing a technology for the production of dietetic burfi for a target group of obese, diabetic and those prone to heart related problems. Studies were conducted for screening of the suitable fat replacers and bulking agents. The necessary process modifications were made for use of these fat replacers and sugar replacers. The critical compositional variables of dietetic burfi including levels of milk fat, fat replacers and bulking agents were optimized using RSM. Aspartame and neotame showed poor stability in dietetic burfi. Sucralose was selected as a high potency sweetener on the
basis of its most preferred sweetness profile and excellent stability in the product. Shelf life studies revealed that vacuum packaged dietetic burfi can be stored without spoilage for 12 days at 30°C and 40 days at 5°C. Arora et al. (2007) reported that use of artificial sweeteners viz. saccharin, acesulfame-K, sucralose and aspartame in burfi resulted in low instrumental hardness, adhesiveness, springiness, gumminess and chewiness with a decreased compactness of the network as revealed by the scanning electron microscopy. Recently, Arora et al. (2010) studied the stability of aspartame in burfi and 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.

Gulabjamun

Gulabjamun is a khoa based sweet popular in India. The traditional method of preparation involves blending of khoa, refined wheat flour and baking powder into a homogeneous mass so as to obtain smooth dough along with small amount of water. The balls of the dough are deep fat fried in ghee or refined vegetable oil to a golden brown colour and subsequently transferred to sugar syrup. Chetana et al. (2004) optimized the critical variable of gulabjamun preparation using sugar substitutes i.e. concentration of syrup, soaking temperature and duration of soaking using response surface methodology. Based on the optimized conditions gulabjamun without sugar could be prepared without affecting the quality of product. Soaking of fried gulabjamun balls in sorbitol syrup of 54°C strength added with aspartame @ 0.25% maintained at 65°C for 3 hrs yielded the good quality product.

Lal peda

Lal peda is one of the most important indigenous dairy products of eastern region of India. It is prepared using khoa as a base material to which about 35% sugar is added during heat desiccation process. Sugar plays a very important role in providing a characteristic texture, reddish-brown colour and caramelized flavor in lal peda. The product is very similar to brown peda (Raju et al., 2008; Londhe et al. 2012). Attempts were recently made by Jain et al. (2013) to replace sugar with permitted high intensity sweeteners (aspartame, acesulfame-K and sucralose) with addition of bulking agents (polydextrose and inulin) to provide a characteristic texture. It was reported that lal peda containing 25% polydextrose and 0.17% aspartame gave an optimum product.

Chhana-based products

Rasogolla

Rasogolla is the most popular chhana based Indian sweetmeat. Because of its pleasant and delightful taste, the fame of this sweet has not only spread throughout India but is becoming popular abroad as well. Quite a considerable quantity of this sweet is now being exported to Middle East and European countries from Bikaner and West Bengal. Because of its high sugar content (32-55%) the people who are suffering from diabetes are not able to relish this delicious product. Technology has been developed for the manufacture of sugar free rasogolla using artificial sweeteners for such a large group of people. The levels of aspartame and sorbitol were optimized on the basis of sensory quality of the product using D6 Hokes design (RSM). 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. Aspartame did not much affect the sensory quality of the product except for its sweetness. No signs of deterioration in terms of flavour body and texture, color and appearance and sweetness of the product were observed up to 20 days at refrigeration temperature and up to 15 days at ambient temperature.
Chhana kheer

*Kheer* is a sweetened dessert made by boiling milk along with rice and sugar to get a semi-solid consistency. Instead of using rice, chhana is used to prepare *kheer* in *chhana kheer*. A process for manufacturing chhana kheer based on milk fat, aspartame, acesulfame-K and sucralose was optimized using response surface methodology by Gautam *et al.* (2013). The formulation with 2% milk fat, 15 mg aspartame, 15 mg acesulfame-K and 5 mg sucralose was considered to be the most appropriate for manufacturing *chhana kheer*.

Fermented dairy products

*Shrikhand*

*Shrikhand* is an acid coagulated indigenous and sweetish-sour, fermented milk product is a popular delicacy in Gujarat, Maharashtra and part of Karnataka. It is consumed as a dessert. This indigenous dairy product is prepared by lactic acid coagulation of milk; separation of whey form curd followed by blending with grounded sugar, flavour, colour and selected spices. It 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.

*Lassi*

*Lassi* is a traditional South Asian beverage, originated in Punjab (India, Pakistan) and made by blending dahi with water, salt and spices until frothy. It is a healthy dairy beverage, the thickness of which depends on the ratio of dahi to water. The product is relished sweet in the northern parts of the country, whereas the salt variety is preferred in the south. 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.

*Misti dahi*

In eastern India, the traditional fermented dairy product, dahi, has been elevated to a dessert by sweetening it. The sweetened variety of dahi is popularly known as misti dahi or misthi doi. *Misti dahi* has creamish to light brown color, firm consistency, smooth texture and pleasant aroma. Various market survey reports on the quality of *misti dahi* sold in different parts of the country revealed wide variations in the 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 develop reduced fat *misti dahi*, Raju and Pal (2009) studied the effect of reduction of milk fat, by keeping the total milk solids constant, and reported that highly acceptable reduced fat *misti dahi* can be produced with 3.0% fat and 15.0% milk solids-not-fat (MSNF). Further, studies were carried out to replace cane sugar in *misti dahi* with a blend of sweeteners along with bulking agents and it was reported that maltodextrin was found to be the most suitable bulking agent in the preparation of artificially sweetened *misti dahi* using a binary blend of aspartame and acesulfame-K (Raju and Pal, 2011). Principal component analysis identified that five components were responsible for 91.59% of the total variance in the quality attributes of misti dahi while the hierarchical cluster analysis revealed two significantly different clusters to indicate difference in
behaviour mainly according to the type of bulking agent used. Any of the three studied sweetener blends (Asp-AcK; Asp-Sucr; AcK-Sucr) with maltodextrin emerged as the best combination (Raju and Pal, 2012). The compositional differences among low-calorie misti dahi are given in Table-1.

![Principal Component analysis biplot of the quality attributes for misti dahi showing PC1 versus PC2](Source: Raju and Pal, 2012)

**Table-1. Composition of conventional vis-à-vis low-calorie misti dahi**

<table>
<thead>
<tr>
<th>Constituent (%)</th>
<th>Low fat misti dahi</th>
<th>Medium fat misti dahi</th>
<th>High fat misti dahi</th>
<th>Sugar-free misti dahi</th>
<th>Dietetic misti dahi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk fat</td>
<td>2-3</td>
<td>4-5</td>
<td>8-9</td>
<td>2-3</td>
<td>2.2</td>
</tr>
<tr>
<td>Milk SNF</td>
<td>13-14</td>
<td>11-13</td>
<td>10-11</td>
<td>11-13</td>
<td>11.4</td>
</tr>
<tr>
<td>Cane Sugar</td>
<td>17-19</td>
<td>17-18</td>
<td>17-18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total sugars</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6-7</td>
<td>16.3</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>5.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total solids</td>
<td>32-35</td>
<td>32-36</td>
<td>35-38</td>
<td>28-30</td>
<td>30</td>
</tr>
<tr>
<td>Calorific value (in Kcal)</td>
<td>145</td>
<td>129</td>
<td>185</td>
<td>79</td>
<td>104</td>
</tr>
</tbody>
</table>

<sup>a</sup> contains inulin as dietary fiber  
<sup>b</sup> contains aspartame and acesulfame-K as sweeteners and sorbitol as bulking agent  
<sup>c</sup> contains aspartame and acesulfame-K as sweeteners and maltodextrin as bulking agent

Source: Raju et al. (2012)
Frozen dairy products

*Kulfi*

*Kulfi* is a popular frozen dessert of Indian origin that occupies a privileged position amongst the traditional Indian dairy products and contains high sugar (13-20%) in it. Technology for the production of artificially sweetened *kulfi* using combination of bulking agents mainly maltodextrin, sorbitol and artificial sweeteners such as aspartame, acesulfame-K and sucralose has been developed. Aspartame was found to be a suitable sweetener with maltodextrin and sorbitol as bulking agents. *Kulfi* mix was flavored with cardamom, filled in mould and frozen in ice and salt mixture. The levels of maltodextrin, sorbitol and aspartame were optimized on the basis of sensory quality and melting rate using CCRD. The level of aspartame had a major impact on sweetness of the product. The body and texture were mainly affected by levels of maltodextrin and sorbitol.

**Conclusion**

With growing evidence of the role of diet and dietary components especially fat and sugar in non-communicable diseases such as obesity, diabetes, cardiovascular diseases etc. worldwide people are cautious of what they eat. With the continuous invention of food additives such as fat replacers and low-calorie and high-intensity sweeteners it has been possible to develop dietetic dairy products that suit the palate of local consumers. Institutes across the country have contributed for the development of low-calorie traditional dairy products such as dietetic *rasogolla, burfi, misti dahi, kulfi, lal peda, chhana kheer*, etc. for the benefit of health conscious consumers in general and calorie conscious consumers in particular. It is not far off for the Indian dairy industry to exploit and reap the benefits of such inventions.

**References**


Introduction

Dairy beverages have been consumed since ages for quenching consumers’ thirst. Most of the dairy beverages fall under the category of fermented foods. These beverages provide a natural base for carrying functional ingredients. Current trends and changing consumer needs indicate a great opportunity for innovations and developments in dairy beverages. Probiotics, prebiotics, synbiotics and associated ingredients add an attractive dimension to cultured dairy beverages. Another potential growth area for dairy beverages include, value added products such as low calorie, reduced-fat varieties and those fortified with physiologically active ingredients including fibers, phytosterols, omega-3-fatty acids, whey based ingredients, antioxidant vitamins, isoflavones that provide specific health benefits.

Most common dairy beverages include varieties of lassi, cereal based dairy beverages, whey based beverages and acidophilus milk among others. The technological aspects of these beverages are discussed in this chapter.

Lassi

Lassi is a traditional beverage having its origin in India and is popular in South Asia in different varieties. The product, as is known conventionally in India, denotes the buttermilk obtained after churning the butterfat from cream or dahi. The product is relished sweet in the northern parts of the country, whereas the sour variety is preferred in the south. Lassi finds mention in ancient Indian scriptures along with its precursor dahi.

Traditional varieties of lassi

Lassi is a traditional South Asian beverage, originally from Punjab (India, Pakistan) made by blending dahi with water, salt and spices until frothy. It is a healthy dairy beverage, the thickness of which depends on the ratio of dahi to water. Thick lassi is made with four parts dahi to one part water and/or crushed ice. It can be flavored in various ways with salt, mint, cumin, sugar, fruit or fruit juice and even spicy additions such as ground chilies, fresh ginger or garlic. The ingredients are all placed in a blender and processed until the mixture is light and frothy. The lassi of Punjab sometimes uses a little milk (to reduce the acid tinge) and is topped with a thin layer of malai or clotted cream. The beverage is enjoyed chilled as refreshing beverage during extreme summers. Saffron lassi, which are particularly rich, are a specialty of Jodhpur (Rajasthan).

In the southern part of the country, lassi is preferred as a salty beverage. It is referred to as ‘buttermilk’, as it is the by-product obtained after churning dahi for buttermaking. This buttermilk is either consumed fresh after salting, or is used as an accompaniment with rice. Several culinary dishes are also prepared from this by-product. While kadhi is popular in the northern and western parts of the country, kaalan varieties are popular in the south.
Lassi has also become quite popular on hot summer days in Turkey, where it typically contains only water, salt, yoghurt and lemon. In areas of the Middle East including Iran and Lebanon, a similar salty yogurt beverage, named doogh, is popular. Sweet lassi is a more recent invention, flavored with sugar, rosewater and/or lemon, mango, strawberry or other fruit juice. During 2002, commercial products resembling sweet lassi began appearing on the U.S. market, with names like Drinking Yoghurt and Yoghurt Smoothie.

**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.

The optimum formulations contained 1.5 kg milk fat, 14.5 kg sugar and 11.0 kg mango pulp; 4.5 kg milk fat, 11.0 kg sugar and 5.5 kg pineapple pulp and 1.5 kg milk fat, 13.5 kg sugar and 6.0 kg banana pulp per 100 kg curd for mango, banana and pineapple lassi, respectively. 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 of fruit lassi was observed with increase in proportion of EPS’ culture up to a certain level. Comparative sensory analysis of mango, banana and pineapple lassi revealed that mango lassi had the highest acceptability followed by pineapple and banana lassi. Thus mango lassi was selected for shelf life extension studies. The study demonstrated that use of Nisin and MicroGARD™ could extend the shelf life of mango 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.

**Traditional Fermented Milk Cereal Beverages**

**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.

**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).

**Kefir**

Kefir is traditional fermented milk, popular in Middle East, having slight acidic taste, natural carbonation, and aroma. The word kefir is derived from the Turkish word ‘Keyif’ which means ‘good feeling’. It is due to overall sense of health and well being when consumed. It is originated in the Caucasus Mountains in the former Soviet Union, in Central Asia and has been consumed for thousands of years. It is a product of fermentation of milk with kefir grains. Kefir grains can be characterized as small cauliflower florets or cooked rice, having a length of 10–30 mm, irregularly shaped, white to yellowish in color, lobed, having firm texture and slimy appearance. These grains are a good source of lactic acid bacteria, acetic acid bacteria and yeast cells and mixture of polysaccharide. The principal polysaccharide is a water soluble substance known as ‘kefiran’. Other yeasts and bacteria that have been recognized in kefir grains are *Leuconostoc mesenteroides, Lactobacillus helveticus, L. brevis, L. plantarum, L. kefiranofaciens, L. kefir, Kluyveromyces lactis, K. marxianus, Saccharomyces lipolytica, Kazachstania aerobia*, and *Pichia fermentans*. Kefir can be made from any type of milk; cow, goat or sheep, coconut, rice and soy but commonly cow milk is
The grains cause its fermentation and produce numerous components including lactic acid, acetic acid, CO₂, alcohol (ethyl 2 alcohol) and aromatic compounds, responsible for kefir's unique organoleptic characteristics: fizzy, acid taste, tart and refreshing flavor.

Fig. 1: Flow-diagram for manufacturing raabdi like milk-cereal fermented beverage
Traditional kefir was made in skin bags that were hung near a doorway; the bag would be knocked by anyone passing through the doorway to help keep the milk and kefir grains well mixed. The traditional, or artisanal, method of making kefir is achieved by directly adding kefir grains (2–10%) to milk in a loosely covered acid proof container which is traditionally agitated once or more times a day. It is not filled to capacity, allowing room for some expansion as the kefiran and carbon dioxide gas produced causes the liquid level to rise. If the container is not light proof, it should be stored in the dark to prevent degradation of vitamins and inhibition of the culture. After a period of fermentation lasting around 24 hours, ideally at 20–25 °C (68–77 °F), the grains are removed from the liquid by sieving and reserved as the starter for a fresh amount of liquid. The temperature during fermentation is not critical as long as it is not above one that will kill the culture (about 40 °C / 104 °F), or much below 4 °C (39 °F) where the process will cease. The grains grow in the process of kefir production, and are reused for subsequent fermentations. Grains can then be dried at room temperature and kept at cold temperature (4°C). For a longer conservation, they can be lyophilized (freeze-dried) or frozen.

The Russian method permits production of kefir on a larger scale, and uses two fermentations. The first step is to prepare the cultures by incubating milk with grains (2–3%), as discussed earlier. The grains are then removed by filtration and the resulting liquid mother culture is added to milk (1–3%) which is fermented for 12 to 18 hours.

Koumiss

Koumiss is similar to kefir but made from mare's milk and the culture organisms do not form grains. It is a beverage where the milk does not coagulate. It is milky gray in color, light, and fizzy, and it has a sharp alcoholic and acidic taste. The main metabolites of fermentation are lactic acid, ethanol, and up to 0.9ml /100ml carbon dioxide. Koumiss's starter culture consist of lactobacilli (*L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus*), lactose-fermenting yeasts (*K. marxianus* var. *lactis*. *Saccharomyces lactis* and *Torula koumiss*), non-lactose-fermenting yeast (*Saccharomyces cartilaginosus*), and a non-carbohydrate-fermenting yeast (*Mycoderma* spp.). Fermentation of koumiss depends on the action of two distinct microbial groups: 1) lactobacilli that are reported to play a major fermentative role affecting aroma, texture and acidity of the product, as well as being of some benefit to human health and 2) yeasts, whose presence is crucial for the desirable properties of carbon dioxide and ethanol. In traditional method, koumiss is produced in smoked horse's hides known as tursuks or burduks, which contain the microflora from the previous season. These containers are filled with unheated mare's milk, and as the Koumiss is consumed, more milk is added to provide an ongoing fermentation. A typical commercial method for the production of Koumiss is based on skimmed cow's milk with added sucrose (2.5g/100g).The milk base is then heated to 90°C for 2-3 min, cooled to 28°C, inoculated with starter culture (approx10 ml /100ml), stirred for 15-20min and incubated at 26°C for 5-6h or until the acidity reaches 0.9g /100g lactic acid.

Conclusion

Beverages are essential part of diet and among them dairy beverages have prime importance by virtue of their nutritional and therapeutic properties. Lassi, cereal based fermented beverages such as bajra lassi, jowar lassi etc., kefir and koumiss among others are popular dairy beverages. Most of the dairy beverages are fermented and also serve as a nutritional base for incorporation of novel functional and healthy ingredients.
References


Introduction

In most of the countries, food is no longer something, which satisfies one’s hunger, but one which promotes health and happiness. Even in developing countries like India, an entirely new range of processed foods has flooded the market like never before. The spurt of activity in processed foods sector has been brought about primarily due to the need for convenience demanded by changing lifestyles. Packaged foods, which ensures not only safety to consumers but also offer an improved quality together with convenience in terms of purchase, storage and consumption, are becoming more and more sought after. Production of ready to reconstitute type of foods will not only increase the shelf life of foods but also they offer numerous advantages to the consumers such as use convenience, time saving, etc. With suitable supplements such products are very useful, especially for the elderly, sick, or handicapped persons, or for people who can devote little time to cooking. Though market for such foods in our country are still limited to urban consumers, defence personnel, tourist and caterers, their demand is expected to increase many folds in near future.

Several traditional fermented products such as dahi, yoghurt, cereal based milk beverages etc. have been prepared in dried form with the purpose of increasing shelf life and use convenience.

Yoghurt Powder

Yoghurt is a highly nutritious protein rich product obtained by fermentation of milk with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The product is highly acceptable to consumers because of its flavor and aroma, mainly attributed to acetaldehyde and its texture. The shelf life of yoghurt is limited to 1 day under ambient condition (25-30°C) and around 5 days at 7°C, which hinders its commercialization.

Improvement of the shelf life of yoghurt can be obtained by lowering its water activity by draining of whey. Another method is drying, e.g. freeze drying, spray drying or microwave drying. Generally, yoghurt is dried by spray drying or freeze drying. Freeze drying is costlier than spray drying but freeze dried yoghurt has better starter counts and flavors in comparison to the latter.

The primary objective of drying is to preserve the yoghurt in a high-quality shelf stable powder form without need for subsequent refrigeration. It would, however, be beneficial if the yoghurt were concentrated before drying to increase its total solids, which improves the efficiency of the drying process; concentration may be done either by removing the whey or by evaporating water from yoghurt.

Spray drying is a well-known process suitable for yoghurt drying because it allows preparation of stable and functional products. However, it has been reported that most of the aroma compounds and rheological characteristics of yoghurt are lost during the spray drying process. The optimum survivals of *L.bulgaricus* and *S. thermophilus* were 51.6-54.7%, respectively, at outlet temperature ranges of 70-75 °C; the final moisture content of the dried product varied from 5.1-6.3% (Bielecka and Majkowska, 2000).
Spray drying is reported to have marked detrimental effects on the flavor of the yoghurt powder (Groux, 1973). Addition of hydrocolloids (carragenan, xanthan and gellan) improved retention of acetaldehyde as well as solubility and dispersibility of spray dried yoghurt (Figueroa et.al, 2002). Significant retention of acetaldehyde was found when yoghurt containing 25% TS was spray dried at 210 and 70 °C as inlet and outlet temperatures, respectively and atomizer speed of 23000 rpm. Dispersibility and solubility indices were not affected much by outlet air temperature (Perez Silva et al., 1997).

Dehydrated Dahi

Though the storage life of curd is much more than that of milk, yet it is a perishable commodity and is rendered unsuitable for human consumption fairly quickly. If dahi can be preserved for use over longer periods by dehydration, it will be a popular ready-to-eat food.

The results of various drying operations tried under different conditions as well as the quality tests carried out are given in Table 1. Out of all drying process employed freeze drying gave the best results. It was seen that the curd, if dried at more than 30 °C, the reconstitution property was very poor. For this reason the other drying methods such as atmospheric and tray drying, spray and infrared drying were found to be not at all suitable for drying of curd. Spray drying, however, found to be better than tray drying operations because of less time of contact with the drying medium (Baisya and Bose, 1974).

Table 1: Drying techniques and quality of dried dahi

<table>
<thead>
<tr>
<th>Drying methods</th>
<th>Drying conditions</th>
<th>Final moisture %</th>
<th>Bacterial count/g</th>
<th>Total titratable acid (% LA)</th>
<th>Reconstitution</th>
<th>Color and flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atmospheric</td>
<td>Drying temp.=55°C Air flow rate=7.1 m³/min</td>
<td>5.4</td>
<td>7 x 10⁴</td>
<td>5.62</td>
<td>Very poor</td>
<td>Turns brown and charred milk flavor</td>
</tr>
<tr>
<td>Vacuum tray</td>
<td>Drying temp.=40-50°C Vacuum=63.5 cm Hg</td>
<td>3.6</td>
<td>5 x 10⁴</td>
<td>5.11</td>
<td>Poor</td>
<td></td>
</tr>
<tr>
<td>Spray Drying</td>
<td>Drying temp.=178-180°C Material flow=50 ml/min</td>
<td>7.5</td>
<td>2 x 10³</td>
<td>6.78</td>
<td>Highly loose texture</td>
<td>Slightly brown and poor flavor</td>
</tr>
<tr>
<td>Infra-red</td>
<td>Drying temp.=30-35°C Voltage=250 volts Air flow rate=6.5 m³/min</td>
<td>5.5</td>
<td>3 x 10⁵</td>
<td>5.06</td>
<td>' '</td>
<td></td>
</tr>
<tr>
<td>Freeze-Drying</td>
<td>Working pressure=0.02 cm Hg</td>
<td>3.4</td>
<td>20 x 10⁷</td>
<td>5.05</td>
<td>Good texture</td>
<td>Excellent color and flavor</td>
</tr>
</tbody>
</table>

Source: Baisya and Bose, 1974
Chakka Powder

Chakka is made from whole or skimmed milk. It is obtained by the removal of whey from curd. It is milky white in color and has soft body, smooth texture and clean but mildly acidic flavor. Chakka serves as the base material for shrikhand making. Its physical and chemical properties greatly influence the quality of the finished product. Owing to high moisture content, the keeping quality of this product is limited both at room as well as refrigeration temperature (Patel, 1982). Drying of chakka was done to increase its shelf life.

Chakka and skimmed milk chakka were dried using an anhydro spray drier (De and Patel, 1989). For drying of chakka the inlet air temperature of 185 ± 10 °C and outlet air temperature of 95 °C were employed. Physical properties of the powder obtained are given in Table 2.

Table 2 Physical properties of chakka powder

<table>
<thead>
<tr>
<th>Type of powder</th>
<th>Bulk density (g/ml)</th>
<th>Particle density (g/ml)</th>
<th>Solubility index (ml)</th>
<th>Dispersibility (%)</th>
<th>Flowability (angle of repose)</th>
<th>Wettability (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standardized milk chakka powder</td>
<td>0.634</td>
<td>1.158</td>
<td>9.0</td>
<td>63.78</td>
<td>55.4</td>
<td>33</td>
</tr>
<tr>
<td>Skimmed milk chakka powder</td>
<td>0.623</td>
<td>1.363</td>
<td>10.0</td>
<td>78.88</td>
<td>47.25</td>
<td>24</td>
</tr>
</tbody>
</table>

Source: Patel and De, 1989

Patel and De, 1989 found that chakka prepared from 4.0 % fat milk produced shrikhand of the best sensory quality. For making chakka powder total solid level in the chakka slurry was optimized. It was observed that the viscosity of slurry increased with increase in TS level. Chakka viscosity of around 526.28 cP at 14 % TS level in slurry and 558.41 cP of 19% TS in standardized milk slurry was found most suitable for drying purpose.

Pearl Millet and sorghum lassi in Ready-to-reconstitute form

Efforts were 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, 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.

**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. 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).

**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).

**Conclusion**

Traditional fermented dairy products offer numerous functional and therapeutic benefits and are an essential part of diet. However, limited shelf life of such products hinders their commercial exploitation. Drying of these products can be carried out with a purpose to increase shelf life and also to provide use convenience. Technologies for drying of yoghurt, dahi, chakka, bajra and sorghum lassi etc. have been developed successfully.
Fig.2: Flow diagram for manufacturing Ready to reconstitute bajra/ sorghum lassi

References:
Introduction

The Indian dairy industry has come long way from dependence to self reliance, as the milk production in the country increased by more than 6folds in the last five decades, from 21.1MT in 1968 -69 to 128.3MT in the year 2012-2013. India emerged as the world leader in milk production in the year 1998 and has continues to hold on to this position even today. The quality of Indian dairy products has so far been a barrier to their entry into the global export market and presents significant risks to consumer safety at the domestic front. The Indian dairy industry in general must bridge the significant quality gaps that exist, meet high quality standards. Indian milk products are classified as (i) concentrated and heat desiccated, (ii) acid and heat coagulated with drained whey, (iii) fermented, (iv) fat rich, (v) frozen and (vi) cereal milk based mixes. A newer generation formulated, convenient, ready-to-use and ready-to-eat traditional dairy products have recently been developed by the Indian Dairy scientists. Irrespective of kind, most of these products are found deficient in quality and safety aspects (Rajorhia, 2006).

Need for food safety management system

Quality of raw material, conditions of transport of milk and the lack of skilled manpower are the major factors the impact quality of the milk products. Lack of control over the quality of raw materials and production process, leading to inconsistent chemical composition and textural properties. The products handled under unhygienic conditions are found to carry high microbiological load. Cases of food poisoning consequent to consumption of inferior quality sweets are reported especially during rainy season and summers. Most products suffer from low shelf-life as a result of total absence of packaging and lack of refrigerated facility during storage, transportation and distribution. About 70% of the marketable milk is handled by unorganized sector and unskilled labors having very minimum knowledge on quality and food safety.

The primary quality requirement of a food product is that it should not harm the consumer when used as intended. The consumers today are increasingly becoming conscious of quality and have started to exercise their right to expect that the food they eat is safe and suitable for consumption. The international market is more demanding in terms of quality, safety and delivery. Codex is connected with the microbiological quality of dairy products and has recommended measures to minimize microbiological contamination. To achieve this, the codex favors the hazard analysis critical control points (HACCP) based approach to enhance food safety. In the current scenario of the Indian dairy industry being the largest milk producer in the world, the implementation of the HACCP quality and food safety management systems would provide it a competitive edge in the international market. There are many Indian food delicacies for which quality standards have not been laid down so far in the food regulations. The Ministry of Health has specified the microbiological quality for major traditional milk products during the year 2005. Food safety systems are of paramount importance on any organization conducting business in the food industry.
Food safety management system ISO 22000:2005

The fundamental purpose of a food safety management system is to assure food safety through the application of a series of management disciplines throughout the food chain. It is particularly intended for application by organizations that seek a more focused, coherent and integrated food safety management system than is normally required by law. The Codex Alimentarius Commission was created by FAO and WHO to develop food standards, guidelines and related texts in 1963. In 1969 The Codex Alimentarius Commission brought out the Recommended International Code of Practice- General Principles of Food Hygiene Good Hygienic Practices. In 2005 The ISO (International Organization for Standardization) stepped in and brought out ISO 22000:2005. Food safety management systems based on HACCP are internationally recognized as the most effective way to ensure food safety and minimize the risk of food poisoning.

Elements of ISO 22000:2005

There are five main clauses that communicate the requirements of ISO 22000:2005 with the first three providing a direct parallel with ISO 9001 and putting in place the main supporting structures together with the need for managerial involvement in the determination of the organisation’s food safety policy, establishing food safety objectives, planning the food safety management system, providing the necessary resources, establishing that the system functions effectively and where necessary identify and implement improvements.

The 5 main clauses are:

Clause 4: Food Safety Management System
Clause 5: Management Responsibility
Clause 6: Resource Management
Clause 7: Planning and realization of safe products
Clause 8: Verification, validation and improvement of the food safety management system.

Role of HACCP and Pre-Requisite Programmes (PRP’s) for effective implementation of ISO 22000:2005

The HACCP is a science based system systematic approach to producing safe food. HACCP is a system that identifies, evaluates and controls hazards which are significant for food safety. It is a
structured, systemic approach for the control of food safety throughout the commodity system, from the plough to the plate. The HACCP system is a dynamic system, capable of accommodating change such as changes in equipment design, processing procedures and technological advancements.

The HACCP system is applied to specific product lines and procedures. In order for the HACCP Plan to be implemented effectively within the establishment it must be based on a firm foundation of Good Manufacturing Practices (GMPs) and other pre requisite programs that effectively control general hazards to food safety (ISO 22000).

**Development and implementation of HACCP plan**

The successful development and implementation of an effective HACCP Plan can be achieved by following the 12 steps and seven principle presented in the below flow diagram.

1. **Assemble Team**
2. **Describe Product**
3. **Identify intended use**
4. **Construct Flow diagram**
5. **On-site verification**
6. **List Hazard Conduct Hazard analysis Determine control measures**
7. **Determine CCPs**
8. **Establish Critical Limit For each CCP**
9. **Establish monitoring System for each CCP**
10. **Establish CA for Deviation**
11. **Establish verification Procedures**
12. **Establish Record Keeping and Documentation**

**12 steps and seven principles for effective HACCP implementation**

There are seven discrete activities that are necessary to establish, implement and maintain a HACCP plan, and these are referred to as the 'seven principles' in the Codex Guideline (1997). The seven principles are:

1. **Conduct a hazard analysis.**
   
   Identify hazards and assess the risks associated with them at each step in the commodity system. Describe possible control measures.

2. **Determine the Critical Control Points (CCPs)**
   
   A critical control point is a step at which control can be applied and is essential to prevent or eliminate a food safety hazard, or reduce it to an acceptable level. The determination of a CCP can be facilitated by the application of a decision tree.

3. **Establish critical limits.**
   
   Each control measure associated with a CCP must have an associated critical limit which separates the acceptable from the unacceptable control parameter.
4. Establish a monitoring system

   Monitoring is the scheduled measurement or observation at a CCP to assess whether the step is under control, i.e. within the critical limit(s) specified in Principle 3.

5. Establish a procedure for corrective action, when monitoring at a CCP indicates a deviation from and established critical limit.

6. Establish procedures for verification to confirm the effectiveness of the HACCP plan.

   Such procedures include auditing of the HACCP plan to review deviations and product dispositions, and random sampling and checking to validate the whole plan.

7. Establish documentation concerning all procedures and records appropriate to these principles and their application

**Identification of safety hazards**

Food safety hazards can be caused by biological (microbiological), chemical or physical agents and cause an adverse health effect. During the identification of hazards, food safety and quality considerations must be differentiated. Presence of allergens and crop contaminants have also found place in safety hazards in recent time.

**Biological hazard**

Most traditional dairy products are at risk from one or more biological hazards that usually originate in raw milk or as a result of poor hygiene during production, handling, packaging, storage and post production. Microbiological hazards like larvae, parasites worms and microorganisms, may enter products from contaminated water, human hands and improperly sanitized utensils. Microbiological hazards include,

- Bacteria (salmonella spp., *clostridium perfringens*, *clostridium botulinum*, *listeria monocytogenes*, *camphylobactor*, *staphylococcus aureus*, *vibrio cholerae*, *bacillus cereus*, etc.
- Viruses (Hepatitis A, Rotavirus)
- Fungi (*Aspergillus flavus*, *Fusarium spp.*)
- Algae

**Chemical hazards**

Chemical compounds are used in milk production and processing chain starting from growing of the raw materials upto consumption of finished products. This may include cleaning chemicals, pesticides, herbicides, toxic metals, nitrites, plasticizers migrating from packages, veterinary drug residues, chemical additives, crop residues, phyllotoxins, bird and animal repellants, rodenticides, wood preservatives and food storage protectors. Allergens and intolerant products can cause allergic reactions within minutes and death within hours. Some food colours, MSG and sulphites can cause reactions similar to allergens. The occurrence and methods of controlling chemical hazards in the traditional dairy products will be discussed.

**Physical hazards**

Like biological and chemical hazards, physical hazards can enter indigenous dairy products at any stage during their production. Physical hazards are normally not found in milk filtered and clarified before use. They may enter the milk products accidentally and may cause illness or injury to the consumers. Physical hazards may include glass pieces, metals, stones, plant leaves, wood, pests, plastic, jewelry, hairs, etc. These hazards can cut mouth and throat, break teeth and cause choking.
Illness would be caused by obstruction of bowel, vomiting or irritation of intestine. Perforation of the gastro-intestinal tract can lead to peritonitis (Rajhoria, 2006).

Pre-requisite programs (PRP’s)

Within a food operation, there are many general activities that are put into place to control food safety throughout the work environment to prevent cross contamination between products and control food safety hazard levels in the product and processing environment. These activities are termed as pre-requisite programmes. Effective implementation of prerequisite programs (PRPs) is essential to establish a sound foundation prior to application of HACCP or other food safety management systems. PRP’s such as GAP, GMP and GHP should be implemented before HACCP is applied. While establishing PRP’s adequate attention needs to be given to the applicable statutory and regulatory requirements. If these PRP’s are not functioning effectively then the introduction of HACCP will be complicated resulting in a cumbersome, over-documented system. Undoubtedly, traditional dairy products may be graded as high risk foods that have a significant likelihood of causing illness or injury to the consumers if they are not properly produced and handled in accordance with the established sanitary operating principles (IRCLASS, 2012). The safety of traditional milk products is guaranteed by good agricultural practices (GAP), good animal husbandry practices (GAHP) and good hygienic practices (GHP). Health of udder and animals, quality of feeds and fodders, milking and milk handling technology, cooling and efficient transport of chilled milk are pre requisite for milk product safety. Accurate testing of milk and milk products, application of HACCP principles and good manufacturing practices (GMP), packaging, storage and distribution would ensure safety. Besides, personnel hygiene should be given importance to minimize contamination of products. In order to build confidence amongst milk producers in pricing, automated methods should be adopted by replacing old fashioned techniques which are vulnerable to human tempering, time consuming, inaccurate and non transparent.

Conclusion

Effective national food control systems are essential to protect health and safety of the domestic consumers. Because of consumers concern for food safety, growing awareness about health and rising disposables income, manufacture of traditional dairy products must be regulated by a set of standards incorporating minimal use of pesticides, herbicides, hormones, veterinary drugs. Consumers are now taking more interest in the way the food is produced, processed and marketed and they are prepared to pay premium price for safe milk products and milk food delicacies of India known for taste and texture. To be successful in market place traditional dairy products must guarantee freedom from contaminants, adulterants and hazardous substances of all kinds.

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IRCLASS, 2012. Food safety management systems lead auditor training course compendium. IRS/TRG/FSMS/LAC/Rev.2


Application of Herbs in Functionality Enhancement of Traditional Dairy Products

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Introduction

Now-a-days the consumers are interested in functional foods which promote health beyond supplying basic nutrition. As a result of this, functional foods are gaining popularity throughout the world and the present food market is flooded with a variety of functional foods. By 2014, the international functional food market is expected to reach a value of about $29.75 billion (Singh et al., 2012). The growing consumer demand coupled with industry interest evidently states that there is a great scope for functional foods in India.

A significant amount of milk produced is utilized for traditional dairy products in India (Pal and Raju, 2007). Indian traditional dairy products have a huge demand and their domestic markets are well established. However, in order to compete and to sustain with the ever increasing functional food market, Indian dairy industry should find ways to induce or to improve the functionality in traditional dairy products.

Herbs and their extracts have a long history of usage as natural remedies for curing many health related complications. Herbs have also found their usage in culinary purposes and some of them have been reported for their use in cheese, yoghurt and other food products. Since 1960, there has been an increased interest in “natural health” and it has propelled the consumption of natural remedies i.e. herbs and their preparations. A considerable portion of today’s functional food market consists of herbal supplemented functional foods (Singh and Hussain, 2011).

Ayurveda. Indian traditional medicinal literature, has prescribed several ways in which the medicinal benefits of herbs could be conveyed via certain foods as carriers. More than 50 medicated ghee formulations made with incorporating different herbal preparations were reported in Ayurveda. However, there is a very little or no literature reported regarding supplementation of herbs into other Indian dairy products to improve their functionality. In the recent past, traditional dairy products have received special attention from the R&D institutions. Research has been carried out to induce and/or to improve the functionality of many Indian dairy products. Developments in the manufacture Indian dairy products with improved functionality, especially herbal supplemented Indian dairy products are depicted here.

Application of herbs and herbal nutraceuticals in milk and milk products

Fat rich dairy products

Herbs contain high amounts of phenolic compounds which possess antioxidant properties. The natural antioxidant properties of herbs have made their use in the formulation of functional foods specifically targeted for the people suffering from cardio vascular diseases (Najgebauer-Lejko et al., 2009). The antioxidant properties of herbs also led their use into fat rich dairy products for retarding auto-oxidation there by prolonging the shelf-life. Moreover, it was found that the artificial antioxidants, like BHT (butylated hydroxytoluene) or BHA (butylated hydroxyanisole) are not safe for human consumption (suspected to have carcinogenic activity). On the other hand, increasing sensitivity of consumers to synthetic ingredients as well as their increasing awareness about the effect of diet on
their health contributed to the increasing trend to use natural additives like herbal extracts for the stabilization of fat rich dairy foods like ghee, butter oil, butter etc. Sage (Salvia officinalis) and Rosemary (Rosmarinus officinalis) extracts are the most widely used for this purpose (Ozcan, 2003). These extracts have antioxidant activity many times stronger than synthetic antioxidants like BHA or BHT (Estévez et al., 2007).

Milk fat, particularly ghee has the characteristics to absorb all the medicinal properties of the herbs with which it is fortified, without losing its own attributes. About 60 medicated ghee preparations used for the treatment of various diseases were reported in Ayurvedic literature (Pandya and Kanwajia, 2002). Recently, Arjuna ghee was developed at NDRI, Karnal by incorporating functional attributes of Terminalia arjuna for providing beneficial effects against cardiovascular diseases and the product was more stable to oxidative deterioration as compared to control ghee (Rajanikant and Patil, 2005). Unlike in case of medicated ghee preparations, Arjuna ghee can be replaced with normal ghee in the daily diet. Pawar et al. (2012) has successfully increased the oxidative stability of ghee by incorporating the alcoholic and aqueous extracts of Satavari herb.

Research evidence supporting the health benefits of herbal ghee preparations is scanty. In a clinical study on antiasthamine effects of vasa ghee (ghrit), Prasher (1999) reported that oral ingestion of vasa ghee was beneficial in reducing the risk of asthama. There was marked improvement in 92.59% cases within 21 days of study period. HPTLC studies have shown that vasicinone, an antiasthamine agent present in Adhatoda vasica was responsible for antiasthamine effects of vasa ghee. The authors have also reported that vasa ghee consumption also had an additional benefit in reducing serum cholesterol level by 30.16%. Pharmaco clinical studies showed that Panchtikta ghee (ghrit) prepared with different methods was beneficial in reducing the cardiovascular diseases (Pandya and Kanawjia, 2002). A thorough study on the bioactive components of herbs and effect of different processing conditions on them during ghee preparation could lead us to diversify the usage of ghee in a well-organized commercial way.

**Dahi and Lassi**

*Aloe vera*, a herb of the Liliaceae family has a long and illustrious history dating from biblical times and given a high ranking as an all-purpose herbal plant. Scientific investigations on *Aloe vera* have gained more attention over the last several decades due to its reputable medicinal properties (Hussain et al., 2013).

*Lassi*, a ready-to-serve traditional fermented milk beverage has got wide popularity in India as well as in overseas markets. Sweet lassi with its characteristic sweet and slightly sour taste can be used as a food carrier for herbal bioactives like *Aloe vera* juice. Hussain et al. (2011) has developed functional lassi using the herb *Aloe vera* (Aloe barbadensis Miller). A culture combination containing NCDC 60 and *Lactobacillus paracse* ssp paracasei L at an inoculum rate of 1 percent was used for functional lassi preparation. Animal study of functional lassi revealed that it has better immunoprotective effects compared to control lassi. Recently, Pal et al. (2012) also supplemented *Aloe vera* juice into lassi to enhance its health benefits. The authors have reported that supplementation of *Aloe vera* juice at 15% level into lassi has obtained optimum sensory scores.

Herbal supplemented probiotic dahi using the herb *Aloe barbadensis* Miller was also prepared by Hussain et al. (2011). The authors have reported that *Aloe vera* supplementation has supported the growth of probiotic strain *Lactobacillus paracse* ssp paracasei L in dahi. The probiotic viability was more than 7 log cfu/ml during 12 days storage period.
Sandesh

Sandesh is a very popular heat-desiccated product of coagulated milk protein mass called chhana. About 80% of chhana produced in Kolkata (West Bengal, India) is converted into sandesh (Aneja et al., 2002). Incorporation of herbs into these kinds of highly demanded dairy products will improve the health status of the consumers. Bandyopadhyay et al. (2007) incorporated herbs such as turmeric (Curcuma longa L.), coriander (Coriandrum sativum L.), curry leaf (Murraya koenigii L.), spinach (Spinacia oleracea) and aonla (Emblica officinalis), separately as a paste, at the 10% level into Sandesh to induce the antioxidant properties into the product. The antioxidative levels of these herbs were compared with the synthetic antioxidants TBHQ and BHA: BHT (1:1) at 100 and 200 mg/kg levels. The authors have reported that the total antioxidative status of herbal sandesh was lower than samples with TBHQ but similar to those with 200 mg/kg BHA: BHT (1:1). The authors have also reported that the use of coriander herb with its antimicrobial and antioxidant properties increased the shelf-life of herbal sandesh up to 8 days at (30±1°C) and 30 days at (7±1°C) when compared with the remaining samples.

Shrikhand

Shrikhand is a semi-soft, sweetish-sour, whole milk product prepared from lactic fermented curd (De, 1980). Shrikhand is prepared by admixing of sugar in required quantities with strained dahi or concentrated dahi. Being a sweetish-sour and semi soft product it can easily harbor herbs/herbal extracts without undergoing significant quality changes. Landge et al. (2011) successfully prepared shrikhand using Ashwagandha herb powder as an additive. The authors have found that addition of 0.5% Ashwagandha powder to shrikhand has improved the organoleptic quality and the product was remained acceptable up to 52 days at refrigerated temperatures.

Other possible uses of herbs to improve the functionality of Indian dairy products

Most of the Indian traditional dairy products contain high amount moisture content besides harbouring valuable nutrients. The high moisture content of these dairy products will favour the growth of microorganisms leading to their spoilage. Phenolic compounds of herbs are a good alternative for the synthetic antimicrobial agents used in food industry. Phenolic compounds namely, ferulic acid, tea catechins, oleuropein, ellagic acid and p-coumaric acid have been reported to inhibit the growth of pathogenic bacteria (Salmonella enteritidis, Staphylococcus aureus, Listeria monocytogenes) and fungi (Schaller et al., 2000). These antimicrobial properties herbs can be effectively utilized to control the growth of unwanted/spoilage and pathogenic microbes in Indian dairy products. Oleuropein derived from Olive tree has been reported to markedly inhibit the production of aflotoxins (Bullerman and Gourma, 1987). This property of oleuropein could be advantageous in products like chhana and paneer where the growth of moulds leading to mycotoxins production may present health risk (Jarvis, 1983).

Conclusion

Herbs are considered as nature’s gift to human beings as they can prevent and cure many illnesses. Herbs harbor a wide variety of functional components which can perform wide range of biological functionalities. In recent past, research regarding functionality of herbal components, toxicology and their use in food products has been the matter of interest. However, depending upon the concentration and type, the incorporation of herbs into food products may have certain undesirable effects on their sensory, physico-chemical and textural properties which in turn could affect their overall acceptability. Presently, the herbal ghee being marketed in the global market is mostly sold as
medicine (medicinal ghee), which is associated with typical flavour, bitter or pungent taste and a dark colour. Such therapeutic preparations are therefore not acceptable for routine use. Incorporation of these nutraceuticals into food systems may therefore calls for technological modifications/alterations so that the sensory quality of the final product remains unaltered.

Furthermore, very limited information is available for ascertaining the residual levels of these functional components in herbal food preparations. Interactions of herbal and food constituents on human health have to be studied thoroughly. More research should be directed towards the effect of processing conditions on the bioavailability of functional components in the herbs so that the processes will be designed in such a way that little or no damage will occur to the functional components during their incorporation into food matrix.

References


Introduction

India is the global leader in milk production with the annual production of 127.8 million tonnes of milk during year 2011-12. It is estimated that the milk production will touch the value of 133 million tonnes in year 2012-13. Currently, the per capita daily milk availability in our country is higher than the recommended level (280 g) of ICMR and may reach to 290 g per day during the current year (Srivastava, 2013).

Our country is known worldwide due to its rich cultural heritage. From ancient time, Traditional Indian Dairy Products (TIDPs) have been considered as a part of traditional, nutritional and economic legacy of the nation. Here, the traditional knowledge to make various sweetmeats from milk has been passed from one generation to next generation, which can be still seen as higher amount of milk is being processed by unorganized sector (i.e. sweet makers or halwais) as compared to organized sector. Historically, the surplus amount of milk has been converted into a variety of these TIDPs in order to preserve original nutritional potent of milk and also to enhance the shelf life of these products at high ambient temperature. Basically, chhana and khoa are the two base/filler materials for a number of chhana and khoa based sweets. Around 7% of India’s total milk production is used to produce six lakh tones quantity of khoa on annual basis (Rajarajan et al., 2007). It has also reported that about 6% of the total milk production is converted to chhana through coagulation (Sahu and Das, 2007). The conversion of milk into TIDPs results in value addition to original milk when compared with its conversion into western dairy products (except malted milk and chocolates), which adds only 50% value to it(Aneja et al., 2002).

The estimated market size of these TIDPs is more than Rs. 1 lakh crores with an estimated annual growth of Rs. 5000 crores. It has also estimated that the market volume of chhana based sweets in India is about 1 million tonnes with a value of Rs. 7,00,000 crores (Sahu, 2007). These TIDPs has been not only delighting the consumers from decades but also maintaining their popularity up to now as different new products are being characterized, standardized and continuously appearing in the food basket of the consumers. Although several TIDPs have been become popular over the time yet there are certain products either recently developed or their production is region specific, can be called as underutilized dairy foods. Different popular and underutilized/regional TIDPs are presented in Table 1.

Technology of chhana based underutilized dairy foods

Kheer Mohan

It is also a chhana based traditional sweetmeat. This product is popular and being manufactured in eastern parts of Rajasthan. It is available in several towns and cities of eastern Rajasthan namely Sawai Madhopur, Kota, Jaipur, Dausa, Karoli but Gangapur city is particularly well-known for its manufacturing. Following are the typical sensory attributes of the product available at Gangapur city:

- Flavor: Cooked/caramelized, moderately sweet, pleasant taste with heated note.
- Body and Texture: Fairly tough, close knit, dense and chewy, granular and non-elastic.
- **Shape and Size:** Round with uneven surface.
- **Color and Appearance:** Medium brown crust with light brown core.

For the preparation of *Kheer Mohan*, buffalo milk is first boiled and allowed to cool for some time. Then the milk is coagulated with suitable coagulating agent, mostly previous day whey to produce *chhana* followed by its mixing with dry sugar and semolina. The mixture of *chhana*, sugar and semolina are then kneaded up to visible fat separation on the palm occurs followed by formation of the *chhana* balls. These balls are then cooked in sugar syrup until desired color is obtained followed by their overnight soaking in sugar syrup. Finally the product is taken out from sugar syrup, stored and sold at ambient temperature.

**Table 1: Different popular and underutilized dairy foods.**

<table>
<thead>
<tr>
<th>Chhana based dairy foods</th>
<th>Khoa based dairy foods</th>
<th>Milk based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Popular</td>
<td>Regional</td>
<td></td>
</tr>
<tr>
<td>Rasogolla, Rasmalai,</td>
<td>Kheer Mohan, Chhuna Jhele, Chhuna Kheer, Chhuna Pakora, Rasaballi, Chhuna Balshai</td>
<td>Kheer/ Payasam, Basundi, Rabri</td>
</tr>
<tr>
<td>Rajbhog, Sandesh,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chhuna-Murki, Cham-Cham,</td>
<td>Gulabjamun, Kalajamun, Pantua, Lalmohan, Burfi, Kalakand, Milk Cake, Peda, DarwadalPeda,</td>
<td>Kharadkhee r, Kashmiri Saffron Phirni, Halvasan, Thabdi</td>
</tr>
<tr>
<td>Chhuna Podo</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Balsahi**

*Balsahi* is one of the most popular *chhana* based sweetmeats of the North Bihar. Slowly, the product is increasing its popularity in other parts of the country. The method of the *Balsahi* preparation is a secret technique of certain sweet-makers. A good quality of *Balsahi* is usually prepared from buffalo milk *chhana* that had hard body and smooth texture.

Recently standardized method for the preparation *Balsahi* has been developed (Prakash et al., 2013). In that study, mixed milk *chhana* (milk heated to 85 °C and coagulated at 80 °C with 1 % citric acid) was broken into smaller pieces and kneaded with 12% suji, 15% sugar to form homogeneous, smooth dough and shaped into round balls of size about 8-10 g. Balls were rolled on palms for 1 min. Care was taken to avoid cracks on the surface. These *chhana* balls then poured into boiling sugar syrup 70% and cooked for 40-45 min. During cooking a small amount of water was continuously sprayed to maintain its concentration. During cooking the balls first settle at the bottom of the pan after a few minutes they start floating on the surface of the cooking syrup. After sufficient cooking of 40-45 min, balls were transferred in a soaking sugar solution of 50% concentration. The product acquired the desired sugar concentration when the equilibrium was reached between the sugar syrup concentration inside and outside of the balls. It required 1-2 h at room temperature. After complete soaking, *balsahi* was stored at or below 8°C.

**Technology of khoa based underutilized dairy foods**

**Kapoor kand**

*Kapoor kand* which is also known as *Bottlegourd burfi* is a *khoa* based traditional sweet popular in certain regions of North and Central India. Standardized method for the preparation of *Kapoorkand*
has been recently developed (Gupta et al., 2010). As reported by the research group, Bottlegourds were first cleaned in running water followed by their peeling, grating and mincing to get Bottlegourd paste. This paste was added in to pre-standardized (5% fat, 9% SNF), partial concentrated buffalo milk (@ 70:30 and 60:40 milk to Bottleguard ratio). During stirring and scrapping of this mixture, 12-16% sugar was added and concentrated until non-sticky consistency of the mixture was obtained. Setting of the mixture then carried out in pre-greased trays followed by its cutting into cubes. Product cubes were then wrapped in waxed coated butter papers and packed in cardboard boxes. Product was made from standardized buffalo milk (5% fat and 9% SNF), Kapoorkand (70:30, milk to Kapoorkand ratio) and 12% sugar observed to have highest sensory scores on five point hedonic scale. Product was then stored at 22-26°C temperature and refrigeration temperature (4-6°C) which obtained the shelf life of 15 and 56 days respectively.

Khoa Jalebi

Khoa jalebi that resembles traditional maidajalebi is a popular khoa based sweet consumed in central parts of India. It possesses thicker coils, light brown to dark brown color and soft yet chewy texture.

The method of preparation of of khoajalebi is similar to that of maidajalebi but differed in ingredients (khoa, arrow root powder and tokir) used. Initially, during preparation of khoajalebi, batter is prepared to right consistency. Khoa and soaked tokir were properly mixed in wet mixing jar for 15-30 sec. Then, the mixture was poured in hot ghee in circular frying pan (27 cm diameter) using a special woven jalebi making cloth (1 cm opening). The batter was given a round shape and one knot and fried for 5 min at medium flame (160-170°C). The fried jalebi was soaked in sugar syrup of 65°C Brix for 5 min (Kumari et al., 2012).

Technology of milk based underutilized dairy foods

Karad kheer

Kheer also known as Payasam is heat desiccated, cereal based highly popular sweetened indigenous dairy product which has nutritional potent of milk and cereal like rice. Kharadkheer, invariably produced from milk and safflower (Carthamus tinctorius) extract, is an indigenous milk product and a traditional preparation of tribal belts of Maharashtra state. As safflower is used as an ingredient in its preparation, it acts as a source of linoleic acid and vitamin F. Standardized method for its production and packaging has not been developed till now but studies on the microbiological quality of the laboratory made product has been reported (Sarode et al., 2007). This research group has prepared product using safflower extract from Bhima variety, 9% cane sugar and standardized milk (SM, 5% fat and 10% SNF) with different combination (i.e. 20 – 100 parts of SM and 80 - 0 parts of safflower extract), finally good quality cardamom with ripe black seeds were added @ 0.3% of the final product. Sodium hexametaphosphate was used @ 0.3% to improve the heat stability of Karadkheer. Most acceptable product was obtained with 60 parts of SM and 40 parts of safflower extract which was further packed in UV irradiated low-density polyethylene (LDPE) bags and Polystyrene (PS) cups, stored at 30°C and 5°C and subjected to microbial analysis up to 15 h time interval. Lowest SPC count was observed in most acceptable product among different combinations while yeast and mold showed uninterrupted growth rate as they were capable to grow in the presence of high sugar and viscous medium.

Kashmiri Saffron Phirni

It is milk based traditional dairy product of Jammu and Kashmir, highly resembles with rice kheer. It is made with semolina, sugar, saffron and dry fruits and their paste is formed in milk to a thick consistency. Recently, an attempt has been made to develop a standardized product with most
preferable sensory attributes (Bhat et al., 2012). Basic ingredients used in its preparation by the researchers were: standardized milk (5.5% fat and 9% SNF, 1 kg), semolina (5, 7.5 and 10% w/v), sugar (10 % w/v), saffron (0.01% w/v) and dry fruits (4% w/v, almond and resins in 2:1 ratio). Different levels of the semolina were slowly added into boiling milk with continuous stirring to prevent lump formation up to ten minutes. Then, into this mixture, sugar and saffron (presoaked in warm milk and reserving a few strands for garnish) was added and simmered with continuous stirring for next 5 minutes. Finally, the product was cooled up to ambient temperature, transferred, packed in LDPE pouches and stored at refrigeration temperature (4±1 °C) for a week. Based on the preferred sensory attributes, best quality product was obtained with 7.5% semolina and 10% sugar level and same product was remained suitable for the human consumption up to 7 days storage period.

**Halvasan**

It was traditionally originated in Kambat/Cambay, an ancient seaport of Gujarat. It is heat desiccated milk based sweet prepared from mixture of milk and sprouted wheat fada (pieces) which has dark brown to brownish yellow colour, sticky, chewy and compact grainy texture.

A laboratory scale process for manufacturing of Halvasan was attempted by Patel et al., (2010). The researchers used mixture containing required quantity of Maida and Fada (sprouted Bhalia wheat, dried at 50-55 °C and grinded wheat flour having pieces of ~ 2-3 mm size) and added it into standardized milk (6% Milk Fat, 9% SNF) at lukewarm temperature. This mixture was heated and maintained at 90 °C for gradual curdling and cooking without lump formation, sticking and burning at the bottom. Coagulated lumps on the surface of the mixture were sprayed with the separated liquid during stirring to avoid any surface drying. On sufficient cooking of fada (spongy texture like a soft junket), it was broken into small lumps by agitation and vigorous boiling. Calculated amount of crystal sugar was added and boiled continuously with intermittent stirring and scraping till the pat formation stage was reached. Required color was then added to the desiccated mass followed by its cooling to the ambient temperature. After cooling and flavoring, ingredients such as nutmeg, cardamom and saffron, etc. were added as required. The required size of pieces ~ 20-25 gm in round flat shape were formed and garnished with chopped pieces of cashew, nut, pistachio, pisti, almond, etc. It was packed under hygienic conditions in required size of boxes with PE liners.

**Thabdi**

Thabdi is a deeply desiccated sweetened milk product produced in Saurashtra region of Gujarat. It has brown colour, caramelized, cooked and ghee like oily flavour, nutty taste and soft grainy texture. The average composition of Thabdi reported by Hirpara et al (2013) was: 11.7 % moisture, 11.6 % protein, 27.7 % fat, 17.9 % lactose, 28.1 % sucrose and 3% ash.

For the manufacturing of this product, standardized milk (0.66 Fat: SNF ratio) was used by research group and after heat treatment 8% sugar on the basis of milk was added at the time of first boiling of milk. The mixture was boiled continuously till pat formation stage. The final heat treatment was given for 40 min. The duration is divided into two parts: 20 min. after pat formation to have graininess development accompanied by addition of ghee at 1.2% and continuing the heat desiccation for further moisture removal and colour and grainy texture adjustment.

**References**


Introduction

India is the largest milk producing country in the world with estimated production of 127.9 million tones (NDDB, 2013). The major portion of milk is converted into traditional dairy products like paneer and paneer-based products, chhana and chhana-based products, khoa and khoa-based sweets and desserts, which are deep rooted in ancient traditions and have a strong cultural heritage. Out of total milk produced in India, 46% is used as liquid milk and milk converted to dairy products include, 27.5% is converted into ghee, 6.5% into butter, 7.0% into curd and 6.5% into khoa (business@mapsofindia.com).

Milk is near-complete and essential food item for all age groups. Dairy products are important sources of many nutrients including calcium, high-quality protein, essential fatty acids, lactose, potassium, phosphorus, and riboflavin. Lactose or milk sugar is the principal carbohydrate in human and animal milk. Human milk contains an average of 7% lactose, while whole cow’s milk contains 4.8%. Lactose is a disaccharide made up of equal portions of two monosaccharides, glucose and galactose.

Approximately 65% of the human population has a reduced ability to digest lactose after infancy because of less or no amounts of Lactase-phlorizin hydrolase (LCT), more commonly known as lactase or beta-galactosidase, which is responsible for cleaving lactose into absorbable monosaccharides, glucose and galactose, in small intestine (Ingram et al. 2009). Lactose intolerance in adulthood is most prevalent in people of East Asian origin, affecting more than 90% of adults in some of these communities (http://ghr.nlm.nih.gov/condition/lactose-intolerance). Consuming small amounts of milk does not exert any adverse effects in subjects with hypolactasia (condition where the availability of lactase enzyme is relatively minimal), though this condition is apparently the most common reason for avoiding milk products by such people (Di Stefano et al. 2002). Since milk is usually one of the most important sources of calcium in one’s diet, people with lactose intolerance who consume less milk and milk products may experience digestion related problems like flatulence, abdominal pain, diarrhea, bloating and nausea.

Fig. 1. Chemical structure of lactose (β form)
Table 1. Approximate lactose content of major dairy products

<table>
<thead>
<tr>
<th>Product</th>
<th>Lactose (%)</th>
<th>Product</th>
<th>Lactose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Milk (Cow)</td>
<td>4.8</td>
<td>Cottage cheese</td>
<td>3.1</td>
</tr>
<tr>
<td>Chakka</td>
<td>4.4</td>
<td>Ice-cream</td>
<td>5.2</td>
</tr>
<tr>
<td>Chhana (Cow)</td>
<td>2.1</td>
<td>Dahi</td>
<td>4.48</td>
</tr>
<tr>
<td>Paneer</td>
<td>2.0</td>
<td>Kheer</td>
<td>8.45</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>0.1</td>
<td>Khoa (Cow)</td>
<td>25.5</td>
</tr>
<tr>
<td>Plain yoghurt</td>
<td>4.7</td>
<td>Shrikhand</td>
<td>1.56-2.18</td>
</tr>
</tbody>
</table>

**Method for lactose reduction**

With the realization that lactose intolerance was prevalent in varying degrees around the world, the development of methods for the preparation of lactose-free product is necessary for lactose-intolerants. Such products can be prepared from either physical removal of lactose (ultrafiltration or protein precipitation) or by hydrolysis of lactose into the constituent monosaccharides viz., glucose and galactose (Dahlquist et al., 1971). With significant progress in this area over the last few years, there are a number of methods available for lactose hydrolysis. Hydrolysis of lactose by acid includes direct acidification of product. Enzymatic hydrolysis of lactose in milk and whey products, preferred over other methods of hydrolysis at industrial level (Ryder, 1988). Enzymatic hydrolysis of lactose by β-galactosidase is one of the most popular technologies to produce lactose-reduced milk and related dairy products for consumption by lactose intolerant people (Ladero et al., 2005).

**Lactose hydrolyzed beverages**

Lactose-hydrolyzed milk is recommended as a strategy for improving lactose tolerance. Lactose reduced milk is prepared at a processing plant by adding the liquid enzyme to previously pasteurized milk and holding for 24 hours. When the appropriate level of reduction has been reached, usually 70%, the milk is pasteurized again to stop lactose hydrolysis. Milk that has 99.9% of its lactose hydrolyzed, labeled ‘lactose free’ is now available on the market. Milk labeled ‘lactose reduced’ must contain at least 70% less lactose than regular milk (FDA, 2013).

Lactose hydrolyzed permeate beverage was prepared by the addition of Lactozyme (*Kluyveromyces lactis*) with an activity of 3000 lactase activity unit per ml (LAU/ml) at rate of 1ml/L at pH 6.5 and incubated at 37°C for one hour to obtain 80% hydrolysis (Suresha and Jayaprakasha, 2004). A fermented drink was made by hydrolyzing lactose in whey using Maxilact (Lactase enzyme from *Kluyveromyces lactis*, DSM Food) at the rate of 0.3 g/L, which resulted in 80% hydrolysis of total lactose (5.1%) in 30 min (Karet et al., 1998). In another study, Paul (1990) hydrolyzed lactose in whey by adding 0.25-1.0 g/L of lactase enzyme.
The effect of incubation time-temperature on degree of lactose hydrolysis in milk was studied by Harini et al., in 2007. Lactose was hydrolysed to 25,50,75 and 100% using enzyme (Lactozym3000L), @ 1ml/L at 40°C and incubating for 30,60,180 and 300 minutes respectively.

Attempts were also made at commercial level by launching beverage with lactose hydrolyzed whey. A Swedish Dairy Co-operative Arla Group used hydrolyzed whey permeate for preparation of a soft drink, which is a mixture of tropical fruit juices, hydrolyzed lactose and whey protein concentrate has been introduced. The product has recently been launched in the UK under the name of ‘Nature's Wonder’ (Anon, 1983).

Fermented dairy products

Some dairy foods such as hard cheeses, cottage cheese and yoghurt contain a lower amount of lactose per serving relative to milk and therefore, cause fewer symptoms of lactose intolerance. During the cheese making process, whey is removed from the curd. Since 94% of the lactose remains primarily with the whey portion, the finished cheese has relatively low lactose content.

Alm (1982) studied the effect of fermentation on lactose, glucose and galactose content in milk and suitability of fermented milk products for lactose intolerant individuals. He concluded that the degree of decrease in lactose in fermented milk products was least pronounced for buttermilk, kefir, and ropy milk but marked for yogurt, acidophilus milk, and bifidus milk. The greatest decrease was from about 4.8 g/100 g (level in unfermented milk) to about 2.4 g/100 g in yogurt on the 11th day. Savaiano and Levitt (1987) conducted a study to evaluate the tolerance to lactose from yoghurt (standardized with different lactose concentration). In this study, ten healthy individuals with impaired lactose absorption were fasted overnight and fed test meals of milk, lactose solution and yoghurt (each containing approximately 20 g of lactose) and 10 g of lactulose solution and found that consumption of yogurt results in a three- to four fold reduction in lactose malabsorption as compared with similar lactose consumption in milk, lactulose and lactose water controls. Yoghurt with active microbial cultures (Lactobacillus bulgaricus and Streptococcus thermophilus) improves the digestion of lactose, which appears to be partly the result of its reduced lactose content, but is primarily due to autodigestion within the intestine by the microbial β-galactosidase enzyme. Frozen yoghurts that have been pasteurized prior to freezing lacks this β-galactosidase activity. According to Martini et al.(1987), lactose indigestion is found to be similar between frozen yoghurt, ice milk and ice cream. However lactase-deficient persons may tolerate significant amount of these products, presumably due to their slower gastric transit time owing to its high solids and/or fat content.

According to the FSSR-2011, dahi or curd means the product obtained from pasteurized or boiled milk fermented by a harmless lactic acid culture or other harmless bacterial culture may also be in conjunction with lactic acid bacteria for souring. Dahi is usually prepared from whole milk containing 11-13% SNF and 4.6-5.2% lactose. During the fermentation process about 10-30% lactose is hydrolysed to its absorbable monosaccharide components, glucose and galactose, giving 4.48% lactose in the final product. Lower lactose concentration in dahi has added advantage over milk for people suffering from lactose intolerance. Dahi products like shrikhand and lassi have still lower amounts of lactose (1.87% and 1.2% respectively) than dahi and thus can be taken by people with hypolactasia.
Indian Dairy products

Lactose was hydrolysed to 25, 50, 75 and 100% using enzyme, and hydrolysed milk was used for khoa preparation. The khoa prepared from lactose hydrolyzed milk has following composition (Table 1)

Table: Chemical composition of Khoa prepared from lactose hydrolysed milk

<table>
<thead>
<tr>
<th>DEGREE OF HYDROLYSIS (%)</th>
<th>MOISTURE (%)</th>
<th>FAT (%)</th>
<th>ASH (%)</th>
<th>LACTOSE (%)</th>
<th>YIELD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.10</td>
<td>21.33</td>
<td>3.52</td>
<td>22.91</td>
<td>17.86</td>
</tr>
<tr>
<td>25</td>
<td>25.79</td>
<td>21.10</td>
<td>3.76</td>
<td>19.25</td>
<td>17.52</td>
</tr>
<tr>
<td>50</td>
<td>24.99</td>
<td>22.24</td>
<td>3.48</td>
<td>11.42</td>
<td>17.01</td>
</tr>
<tr>
<td>75</td>
<td>24.73</td>
<td>22.38</td>
<td>3.27</td>
<td>9.32</td>
<td>16.98</td>
</tr>
<tr>
<td>100</td>
<td>21.72</td>
<td>23.14</td>
<td>3.04</td>
<td>0.00</td>
<td>16.47</td>
</tr>
</tbody>
</table>

(Source: Harini et al., in 2007)

Compare to the control samples, khoa prepared from lactose hydrolyzed milk has higher HMF content (around 6 to 6.5 times), higher acidity (2- 10%), and high penetration depth (upto 4 times). HMF, acidity and penetration depth were found increased with degree of lactose hydrolysis.

Sharma and Reuter (1991) had prepared good quality chhana using skim milk ultrafiltered-diafiltered retentate and plastic cream. Skim milk was heated to 95°C/5 min, it was then ultrafiltered (26% TS), followed by diafiltration of retentate (23% TS) with equal amount of water to reduce the lactose content. Pal et al. (1994) prepared a base for rasogolla mix powder in which cow’s skim milk was ultrafiltered to about 3-fold concentration to achieve a product containing all the milk proteins along with a part of minerals and lactose. To further reduce the level of lactose to almost the same level as in chhana, UF retentate has to be diafiltered. Sachdeva et al. (1993) produced good quality lactose-reduced paneer using ultrafiltration. The process involved standardization and heating of milk followed by UF, whereby lactose, water and some minerals were removed.

Conclusion

Lactose intolerance, whether inherited or acquired, causes individuals to reduce or eliminate milk and milk products from their diet. However, low intake of milk and milk products has been shown to increase the risk of osteoporosis, hypertension, and some forms of cancer. To combat such problem people have either shifted to milk products containing less lactose content or processes have been developed for the production of traditional Indian dairy products with less lactose content. Research has shown that most of the fermented dairy products have reduced lactose content than other milk counterparts. Processes for traditional products like paneer, khoa, chhana etc. which contains reduced lactose and minerals content have been developed. Further research is required in this area to develop new dairy products with low lactose content or to develop other innovative modifications for
traditional Indian products such that they are suitable for consumption by people with lactose intolerance.

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Fortification of Indian Dairy Products with Functional Ingredients

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Introduction

Functional foods are defined as ‘foods that contain some health-promoting component(s) beyond traditional nutrients’ (Berner and O’Donnell, 1998). They also go by the synonyms of nutraceuticals, designer foods, medicinal foods, therapeutic foods, superfoods, foodiceuticals, and medifoods. Foods can be modified by addition of phytochemicals, bioactive peptides, ω-3 polyunsaturated fatty acids and probiotics and/or prebiotics to become ‘functional’ (Berner and O’Donnell, 1998). Some examples are listed in Table 1. The functional food market is growing at a rate of 15–20% per year, and the industry is claimed to be worth $33 billion (Hilliam, 2000). India is a booming sector for nutraceutical products since 2010, owing to the Commonwealth games organized in Delhi in 2010, which exposed Indians to top class sports and athletes. Growing awareness about health and wellness in the country is another contributing factor to this boom. This market was US $ 1.48bn in 2011 and is expected to grow at an annual rate of 13% to US $ 2.73bn in 2016. Although beverage premixes and ready to drink formulations are the fastest growing area of the country’s nutraceutical segment, consumers in India prefer to mask the taste of such supplements with their regular food. Hence, localisation and fortification of traditional food are two focal points in capturing the Indian market. Fortification of traditional dairy products of India, although in its infancy, has woken up and taken the first few steps into its foray into the industry.

Fortification with whey

Lassi-like cultured milks containing whey and buttermilk were developed to explore the potential demand of lassi and also to utilize these dairy byproducts in a profitable manner. Several workers have reported different raw materials, additives and treatments used for the purpose.

Bambha et al. (1972) developed Whevit, a fermented whey drink. Paneer or chhana whey was separated, steamed, cooled to room temperature and filtered. Sugar syrup, citric acid and colouring were added to the filtered whey, which was then inoculated with Saccharomyces cerevisiae and incubated at 22°C/14 to 16 h. Artificial flavours used were orange, pineapple, lime and mango. Gandhi (1989) patented Acidowhey – a lactic-fermented non-carbonated beverage produced from cheese or paneer whey using Lactobacillus acidophilus culture at 2% level. Singh et al. (1994) formulated whey-based beverages using paneer whey, cheese whey and various fruit juices (mango, pineapple, lemon and banana), at levels ranging from 73 to 87% and 5 to 20%, respectively. Among the beverages, mango flavour scored the highest. It contained 15% mango pulp, 78% paneer whey, 7% cane sugar and had a pH of 4.5. Khamrui (2000) prepared a ready-to-reconstitute kinnow mandarin juice-whey beverage after concentrating the juice to 23% total soluble solids and Cheddar cheese whey to 45% TS by reverse osmosis. They were blended with other ingredients such as sugar, stabilizer mixes and colouring and flavouring ingredients. Final formulation of beverage comprised of 40% kinnow juice and 53% whey in addition to other ingredients. Kumar (2001) developed a flavoured whey-milk beverage containing 70-80% whey by admixing cheese or paneer whey with buffalo milk.
**Lassi** using buttermilk and soybean was made by Deka et al. (1984). The blanched soybean cotyledons were ground with buttermilk to get slurry with a soy-solids-buttermilk solids ratio 2:1. The slurry (12% TS) was homogenized (175 and 35 kg/cm² at 65°C), pasteurized (85°C) and inoculated with *Lactobacillus bulgaricus* + *Streptococcus thermophilus* @ 2%. After incubation at 37°C for 10 to 12 h, *dahi* was sweetened with sugar syrup and thoroughly mixed. The resultant *lassi* had 10 days storage life at 5°C in polyethylene pouches.

*Lassi*-like beverage was developed using *paneer* whey and buffalo milk with pectin and CMC as stabilizers. After neutralization, *paneer* whey (pH 6.6) was mixed with standardized buffalo milk (6% fat), followed by addition of stabilizer, heating (80°C), cooling (30°C), inoculation with NCDC167 culture @ 1% and incubation at 30°C per 14 to 16 h. *Dahi* was then blended along with sugar syrup and flavour. The beverage had 1.35% protein, 19 to 36% TS, 1.9% fat and 4.0% lactose. The product with 70% whey and a combination of pectin and CMC 0.6% level was adjudged most acceptable (Mittal, 2003). Kumar (2004) developed *lassi*-like beverage from rennet whey. It contained 67.27% whey and standardized buffalo milk (6% fat), followed by addition of a stabilizer mix of pectin, CMC and trisodium citrate, hydration for 30 min, heating (80°C/10 min) and cooling (30°C). Starter was added @ 2% and the mixture was incubated at 30°C for 12 h. The coagulum was blended after the addition of cooled sugar syrup and flavor. The beverage had 2.0% fat, 1.85% protein, 12.49% sucrose and 4.54% lactose. Its acidity and pH were 0.675% LA and 4.24. The *lassi*-like whey-based beverages developed were subjected to UHT-treatment. The final results suggested that milk could be replaced by 70% *paneer* or cheese whey in the preparation of a *lassi*-like beverage, thus ensuring complete utilisation of large quantities of whey. The product is also amenable to UHT-treatment and has a shelf life of over six months.

**Protein enrichment**

Malik and Kempanna (2011) studied the quality characteristics of yoghurt enriched with spirulina powder. Yoghurt prepared with 0.3% spirulina had comparable scores for sensory parameters with that of control. The viability of yoghurt culture declined whereas acidity and soluble nitrogen content increased during storage at 4°C prepared with 0.3% spirulina and had a good shelf life up to 15 days at 4°C.

**Dietetic traditional dairy products**

Most of the traditional Indian dairy products contain high fat and high sugar. Being aware of the impact of high intake of fat and sugar on health, today’s health conscious consumer is looking for low or reduced calorie traditional dairy foods. Chetana (2004) reported that *jamuns* soaked in sorbitol syrup having 45° Brix (B) were soft, while those soaked in 55° B were highly acceptable and those containing 65° B were chewy and a hard core center. Syrups prepared with bulking agent maltodextrin + polydextrose along with the intense sweetener aspartame showed lower overall acceptability score. Milk *burfi* could be prepared with the quality characteristics similar to that of *burfi* with sugar using sorbitol and mixture of sorbitol and mannitol (90:10). Chavan et al. (2009) had prepared dietetic and diabetic *rosogolla* with acceptable quality, using *chhana* made from cow milk standardized to 2% milk fat. Well kneaded *chhana* balls can be cooked and soaked at 40° B double refined sugar solution to obtain dietetic *rosogolla*, whereas 40° B sorbitol solution is required for cooking of diabetic *rosogolla* followed by soaking in 40° B sorbitol solution containing 14.3 g/L aspartame. Arora et al. (2010) successfully replaced sucrose with aspartame at a level of 0.065% of milk (w/w) which scored highest in terms of sweetness perception and resembled control *burfi* in sweetness. Raju and Pal (2011) used a blend of aspartame and acesulfame-K along with different bulking agents to completely replace the cane sugar in *misti dahi* and concluded that maltodextrin is
the most suitable bulking agent. Jain et al. (2013) replaced sugar with artificial sweeteners (aspartame, acesulfame-K and sucralose) with the addition of bulking agents (Litesse and inulin) to provide a characteristic texture in lal peda, a traditional Indian heat desiccated dairy product. Lal peda prepared using 25% Litesse and 0.17% aspartame gave an optimum product. Gautam et al. (2013) studied the sensory and textural properties of chhana kheer made with three artificial sweeteners. Aspartame and acesulfame-K at the level of 0.015% and sucralose at the level of 0.05% were found to be the most appropriate levels for chhana kheer replacing conventional product.

Shuwu et al. (2011) developed value added lassi using honey. Among the four honey lassi samples evaluated by sensory analysis viz., 27.5, 30, 32.5 and 35% honey syrup level, the sample at 30 per cent honey syrup level secured highest overall acceptability score of 8.20. Three different fruits pulp (mango, pineapple and banana) were used at 7% (w/v) pulp level to develop fruit lassi, pineapple lassi secured the highest overall acceptability score (8.33) followed by mango and lastly the banana sample. The physico-chemical analysis revealed a significant increase in TS (18.93 and 18.65%), carbohydrates (17.74%) and slight decrease in protein (2.1 and 1.98%) and ash content (0.45 and 0.46%) and significant decrease fat (2.0 and 1.5%) of both the honey and pineapple lassi respectively against the control sample (17.78% TS, 2.2% protein, 2.5% fat, 17.4% carbohydrates, 0.51% ash content).

Fortification with dietary fiber

Milk and most dairy products are devoid of dietary fiber. Indigenous dairy products that contains significant amount of dietary fiber are gajar-halwa, ghia-halwa, kaju and doda burfi. Composite dairy cereal based kheer and dalia are other traditional dairy products with high amount of dietary fiber. Kantha (2005) developed a low fat paneer using soy fiber and inulin and reported that milk containing 2.5% fat and 0.56% soy fiber or 1.8% fat and 4.5% inulin yielded a paneer similar to that prepared from full cream milk (6% fat) in terms of sensory characteristic. Recently whey and fermented milk products like dahi, lassi, shrikhand have been fortified with fruits to increase the dietary fiber content, simultaneously giving other health promoting benefits. Kanawjia et al. (2011) developed a technology for manufacture of bajra lassi (milk-pearl millet based fermented beverage) using milk solids and pearl millet. The bajra lassi contained 9.74% TS, 2.3% fat, 2.5% protein and 1.28% ash (including salts and spices). The shelf life is 7 days at refrigeration temperature packed in LDPE pouches. Addition of potassium sorbate and nisin further increased the shelf life from 7 days to 35 and 28 days respectively. Meshram et al. (2011) used orange concentrate in the preparation of shrikhand. Taking in to consideration both cost structure and overall acceptability, shrikhand prepared with 3.5% orange concentrate was found superior. Verma et al. (2011) investigated the quality of yoghurt prepared from cow milk blended with beet root and tapioca powder. Beet root is a rich source of potent antioxidants and nutrients including vitamins, minerals which are important for the cardiac health. The beet root yoghurt was prepared from cow milk standardized for 4% fat and 13% SNF using cow cream and SMP and cow cream along with admixture of SMP and tapioca powder in the ratio of 3:1. The levels of beet tried were 27 and 5% respectively. Shrama and Choudhary (2011) studied the effect of processing on the sensory and nutritional quality of fenugreek leaves incorporated paneer. Blanched (hot water), chopped and before coagulated incorporated fenugreek leaves were most acceptable and nutritionally high in terms of protein, calcium, fiber, iron and vitamin C. Divya and Kumari (2009) developed a soft beverage from paneer whey and guava pulp which pasteurized at different temperatures and timings for estimating its shelf-life. In the preparation of beverages the volume of guava pulp (25%), sugar (10%) and paneer whey (65%) were kept constant while the pasteurization temperatures and timings were varied from 60°C-70°C for 15-35 minutes. whey-guava beverages pasteurized at 70°C for 35 minutes was found to be best in terms of
sensory quality after 45 days and pH, acidity, protein, total sugars and reducing sugars found to be high than that of the other samples. Kumari and Dubey (2011)\(^6\) developed a whey based amla and pudina beverage and found that beverage containing 40% amla and 10 ml of pudina extract were scored highest for overall acceptability and were best on the grounds of protein content and total solids. Gaikwad et al. (2011)\(^7\) prepared chhana whey beverage using sapota pulp and the results shows beverage containing 5% of sapota pulp by weight of chhana whey has the maximum overall acceptability score of 8.03. The values of moisture, fat, protein, ash, total solid and carbohydate of the optimized product was 92.51, 0.51, 0.40, 0.51, 7.49 and 6.07 percent respectively.

**Dairy products for heart health**

High cholesterol is one of the major causes of heart diseases. Fat rich dairy products like ghee, butter etc. is not only high in saturated fatty acids but also in cholesterol, therefore there is ample opportunity for dairy food formulators to develop variety of traditional dairy product for heart healthiness. Incorporation of herbs and plant extracts containing phytosterols not only lowers the low density lipo-proteins but also helps in the enhancement of high density lipo-proteins.

Rajnikant (2005) developed arjuna ghee using 60% fat cream, skim milk and arjuna bark. The product possessed similar sensory characteristics of market ghee. The arjuna ghee was found to be highly stable for (8 days 80°C) as compared to control ghee (2 days at 80°C) due to presence of antioxidants like polyphenols, terpenoids and phytosterol contributed by the arjuna extract. The arjuna contained 0.0004-0.035% phytosterol. Parmar (2012) developed an improved process of herbal ghee production from buffalo milk using arjuna extract. The optimized product was obtained by addition of 7% alcoholic arjuna extract using creamery butter method and contains 0.39 mg/g phytosterol. Maurya (2012) develop a technology of curcumin fortified lassi with enhanced health attributes. The optimized product contains 250 ppm of curcumin added with 750 ppm β-cyclodextrin as carrier material.

**Conclusion**

In order to classify as potential ingredients, nutraceuticals have to establish their efficacy and safety like any other healthcare product. There are no mandatory multiphase clinical trials required. Historically, in India multiple laws and regulation were prescribed which possess varied standards regarding food, food additives, contaminents, food colors, preservatives and labeling. India has passed Food Safety and Standards Act in 2006 which is a modern integrated food law to serve as a single reference point in relation to regulation of food products including nutraceuticals, dietary supplements and functional food. The FSSA needs to be made substantive with infrastructure and appropriate stewardship for it to match with international standards of U.S. and Europe.

**Selected readings**


### Table 1. Modification of foods by adding functional ingredients

<table>
<thead>
<tr>
<th>Ingredients added</th>
<th>Reported functionality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytochemicals (as plant ingredients or extracts)</td>
<td>Antioxidant, lower risk of CHD, lower risk of cancer, lower blood pressure</td>
</tr>
<tr>
<td>Bioactive peptides</td>
<td>Enhanced immune function, enhanced bioavailability of minerals, hypotensive</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>Prevention of constipation, lower risk of colon cancer, lowering of blood cholesterol</td>
</tr>
<tr>
<td>ω-3 polyunsaturated fatty acids</td>
<td>Lower risk of heart attack, lower risk of some cancers, enhanced immune system</td>
</tr>
<tr>
<td>Probiotics</td>
<td>Improved gastrointestinal function, enhanced immune system, lower risk of colon cancer</td>
</tr>
<tr>
<td>Prebiotics</td>
<td>Improved gastrointestinal function, enhanced immune system, lower risk of colon cancer</td>
</tr>
</tbody>
</table>

Source: Berner and O’Donnell (1998)
Introduction

Changing demographics and life styles are leading to changes in food habits of the consumers. Food is often consumed away from home thereby increasing the use of processed foods. Rapid urbanization, increase in disposable incomes, and emergence of nuclear families coupled with increase in the percentage of adult women working, have very significantly impacted food preparation and consumption patterns. Today's consumers have little or no time to prepare food at their homes. Consumers are outsourcing food preparation thus emphasizing the need for prepared foods very often in the form of convenience foods. In India with the rapid growth in personal incomes, the expanding middleclass has begun to demand convenience foods. A recent survey conducted by the Confederation of Indian Industry also suggests that demand for ready-to-eat foods is growing at the rate of 20% (FICCI, 2006).

In India nearly 56 per cent of the milk produced is available as marketable surplus for urban areas and a fairly large proportion of it is converted into traditional milk products. Indian dairy products play a significant role in the socio-economic and religious activities of our population. The market for traditional dairy products is valued at about more than Rs.400 billion which states that there is a tremendous potential for this sector (Patil and Singh, 2005). With a large domestic consumer base and continuous rise in ethnic population throughout the world, there exists considerable growth in demand for these products. The changing global scenario and globalization offers Indian dairy product sector an opportunity to become a global player to command the ethnic food markets. It is therefore essential that research priorities in the country should focus on greater value addition for imparting competitive edge to the Indian dairy industry for meeting the emerging challenges of the domestic and global market. Manufacture of Indian traditional dairy products in a convenient to eat or ready-to-reconstitute form not only satisfies the growing consumer demand for these products but also boosts the Indian dairy industry to new heights in the global market.

Keeping in view of the consumers demand for the value added foods, several ready-to-reconstitute formulations have been developed at National Dairy Research Institute, Karnal and its southern regional station Bangalore. The present article describes several ready-to-reconstitute traditional dairy product formulations developed in India.

Ready-to-reconstitute traditional milk desserts developed at NDRI, Karnal

Several ready-to-reconstitute traditional dairy desserts were developed at NDRI, Karnal. Their technology can be readily transferred to prospective manufacturers. Some of these are delineated below.

Ready-to-reconstitute Rasmalai mix

Preparation of ready-to-reconstitute rasmalai mix is a combination of two different processes wherein syrup mix powder and dehydrated patties were prepared separately and mixed together (Mishra et al., 2004). The preparation of syrup mix powder consisted of dry blending of sweetened milk solids (SMS) powder, whole milk powder (WMP) and sugar powder. Saffron was also added to the dry mix before packaging in co-extruded multilayer plastic pouches. The SMS powder was prepared from standardized buffalo milk (fat: SNF ratio of 0.35) by heat desiccation. Standardized milk was boiled in a steam-jacketed stainless steel scraped surface heat exchanger to obtain sweetened khoa with a smooth body. The mixture was transferred into a stainless steel tray and allowed to set for 12 h at room temperature. The resulting pat was cut into cubes and dehydrated using a novel technology to a moisture content of less than 10 percent. The dried cubes were ground into a fine powder.
Dehydrated patties were prepared with standardized cow milk (fat: SNF ratio of 0.35) *chhana*. The *chhana* balls flattened to patty shape were cooked in the sugar syrup and subsequently partially dehydrated by osmotic dehydration technology till the moisture content was less than 10 per cent. The dehydrated patties were vacuum packed in co-extruded multilayer plastic pouches.

For rehydration, the syrup mix powder (100 g) was poured into a bowl containing 150 ml of water and made into a smooth creamy paste. Measured volume of water (250 ml) was separately taken into a vessel, and brought to boil. The patties were then put into the boiling water and rehydrated for 2 min. Subsequently, the creamy paste was added to the vessel, and boiling continued for further 2 min. The product was then to be brought to room temperature, garnished and served cool. It was demonstrated through storage study data that the ready-to-reconstitute *rasmalai* mix was acceptable even after 4 months of storage at 30°C.

**Ready-to-reconstitute Basundi mix**

Ready-to-reconstitute *basundi* mix was also developed using the concept of osmo-air drying and spray drying (Sharma et al., 2004). The product mix was formulated by dry blending of ingredients like whole milk powder (WMP), sweetened milk solids (SMS), particulated whey proteins (PWP) and sugar.

The spray dried WMP was prepared from mixed milk standardized to a fat: SNF ratio of 0.27. The SMS powder was prepared from standardized buffalo milk (fat: SNF ratio of 0.35) by heat desiccation. Particulated whey protein (PWP) was made by coagulating heated whey-and-cow milk mixture with a food grade coagulating agent and subsequently admixing with partially desiccated sweetened milk solids in predetermined proportion. The entire mixture was transferred to a stainless steel tray and allowed to set for 12 h at room temperature. The resulting pat was shredded and air dried to get desired flakes. Dried shreds were packaged separately in co-extruded multilayer plastic pouches.

For rehydration, two hundred milliliters of water was taken into a stainless steel vessel and brought to boil. The entire content of the plastic pouch containing the dry *basundi* mix (powder) was then added with continuous stirring followed by cooking on a low flame for five min. The plastic pouch containing flakes was then opened and the content added into the boiling liquid. After about half a minute it was garnished with dry fruits. The product was then cooled and served. Storage study revealed that the product was acceptable after four and half months at 25°C.

**Khoa Powder**

Ranganadham (1988) developed technologies for small scale and industrial production of *khoa* powder. Three different approaches were tried for the manufacture of *khoa* powder at small, medium and large scale. In the first approach, *khoa*, made from standardized buffalo milk by traditional method was grated into flakes and subjected to heat treatment to evaporate moisture before grinding in a small scale laboratory grinder. The ground *khoa* was uniformly distributed over an aluminium tray and dried in a vacuum and atmospheric hot air oven at 70°C. In the second process, grated *khoa* was dried in a fluid bed drier with an inlet air temperature of 98°C. It took about 4 hours to dehydrate the product in hot air oven and 30 min in fluid bed drier.

The drum drying process was standardized for medium scale operation. Buffalo milk was adjusted to 6 percent fat and 9 percent SNF and heat treated to develop a typical cooked flavour in the final product. The heated and partly concentrated milk was drum dried after adjusting the steam pressure, flow rate of milk and speed of roller drums. Spray drying technology was considered suitable for large scale production of *khoa* powder. Concentrated milk with 30 percent total solids was prepared from standardized buffalo milk followed by heat treatment to develop cooked flavour. The heated, concentrated milk was instantly dried in an Anhydro spray drier with an inlet temperature of 190°C and outlet temperature of 78°C.

On reconstitution with water, this can be utilized directly for the preparation of *burfi*, milk-cake, *kalakand* and *gulabjamun*. *Khoa* powder packaged in tin containers under nitrogen gas can be stored for up to 10 months at 30°C.

**Rasogolla mix powder**
Pal et al. (1993) successfully developed *rasogolla* mix powder by employing ultrafiltration process. Ultrafiltration of cow skim milk was carried out to obtain a 3 fold concentration. Later, diafiltration was carried out to remove the excess minerals, lactose and protein present in the retentate so that it attains a composition similar to *chhana*. The pasteurized cream was added to diafiltered retentate and subsequently sprays dried under standard drying conditions. The dried retentate was blended with selected additives to produce desired flavour and texture.

For manufacture of *rasogolla*, an equal quantity of water was mixed to the mix powder and held for about 5 min for rehydration of proteins. The *chhana* dough was shaped into circular balls of about 7 g size in a manner such that no cracks appear on the surface. The balls were first cooked in sugar syrup of 60% concentration for 15 min and later transferred into hot sugar syrup of about 40% concentration.

**Gulabjamun mix powder**

Two formulations of *gulabjamun* mix powder (GMP) were prepared by (Gosh et al., 1986). The GMP, based on roller dried skim milk powder (SMP), consists of SMP, maida, semolina (suji), dalda (vegetable fat), baking powder and cardamom in definite proportions. All the ingredients were dry blended in a power driven mixer such that the ingredients were uniformly mixed. The vegetable fat was added in molten state intermittently to ensure thorough mixing with the dry ingredients. The GMP packaged in laminate pouches and stored in dry place remains acceptable upto 6 months at room temperature.

Use of spray dried SMP in place of roller dried SMP was not found acceptable as it led to case hardening of balls during deep fat frying and prevented the sugar syrup penetration thereby resulting in unacceptable product. However certain alterations in the ingredients viz., use of high heat SMP, increase in fat content from 15% in roller SMP based formulation to about 18% in spray based SMP, use of additives such as CMC and sodium citrate and/or addition of dried whey protein concentrates (1-2% of SMP) to the mix, helped in improving the textural properties of the product and resulted in highly acceptable product. However GMP based on spray dried SMP requires certain modifications in manufacturing procedure such as increase in the holding time of dough and frying at slightly lower temperature. The shelf life of spray dried SMP based formulation was comparable to GMP based on roller dried SMP (Gosh et al., 1986).

Process has also been standardized for manufacturing *gulabjamun* from GMP. The process consisted of preparation of *khoa*-dough of suitable consistency (50-55 ml water for 100 g mix), which could be made into smooth balls of uniform size and shape and deep fat fried in vegetable oil (Dalda) at about 125°C for 15-20 min. The properly fried balls acquired brown colour which were subsequently transferred into hot sugar syrup of known brix. Even when SMP and vegetable fat were completely replaced with whole milk powder in the GMP formulation, product characteristics remained largely unchanged.

**Instant Kheer mix**

Jha (2000) prepared instant dry *kheer* mix with good reconstitution properties. The process for preparation of instant *kheer* mix consisted of spray drying of admixture of milk concentrate and rice flour (partially preheated to pre-gelatinize the rice starch) along with sugar followed by fluidized bed drying to improve the reconstitution properties. Rice grains which could be readily rehydrated were obtained by a technology which involved partial cooking of rice followed by conversion into a paste, subsequent extrusion and dehydration in air dryer. This form of instant rice was rehydrated in about 5 min. Alternatively, quick cooking rice was obtained by drying of partially cooked rice in a fluidized bed dryer. The rice thus obtained could be cooked in hot water in about 10 min. Spray dried *kheer* powder mixed with instant rice was packed in metallized polyester laminates and the product was stable up to 6 months at room temperature.

**Instant Kulfi mix**
Manufacture of kulfi mix powder (Ghosh, 1991) involves preparation of a mix from milk fat (11%), MSNF (16%), sucrose (15%) and isabgol husk (0.2%). The total solids concentration of the mix was adjusted and only 25% of the total sugar required in the final mix was added before drying. The mix was homogenized at 6.83/3.43 MPa, heated at 100°C for 10 min in a tubular heat exchanger followed by cooling to 4°C. The mix was spray dried. The remaining sugar in ground form was dry blended with the powder and packaged in tin cans. The product has a shelf-life of 7 months at 30°C in tin cans. Kulfi mix powder can be instantly reconstituted and frozen to get kulfi of consistently good quality all the year round at an affordable price.

**Instant Dalia Mix**

Dalia is milk and wheat-based particulate containing dairy dessert which is popular as a breakfast food in India. Limited keeping quality of dalia hindered its commercial manufacturing and marketing. In order to promote dalia dessert as a marketable product, a process has been developed for manufacture of instant dalia mix, as a dry product with long shelf-life (Jha, 2006). The product development consisted of powdered liquid phase and particulate phase. Milk concentrate along with added wheat flour and sugar was dried in two-stage spray drier with integrated fluidized bed drier for agglomeration of the product (powdered liquid phase). The particulate part consisted of instantized dalia made by precooking whole wheat grains for pre-gelatinization and drying the cooked grains in fluidized bed drier and cracking them into dalia. The product so developed was packed in PE-Paper board cartons.

**Ready-to-Reconstitute traditional milk desserts developed at NDRI, Bangalore**

**Palada payasam dry Mix**

The name palada payasam given because of the two main ingredients used in its manufacture, pal i.e. milk and ada i.e. rice flakes. The product is less stable and its organoleptic quality deteriorates within few days of its manufacture. A method for preparation of palada payasam dry mix with good shelf life at room temperature was commercialized by Unnikrishnan et al. (2003). In this method, ada flakes (prepared using rice flour) were boiled in water followed by soaking for about an hour. With occasional stirring, water was decanted and the soaked ada was washed 2-3 times with cold water. Later, milk and sugar were added and heated in a steam kettle to a pasty mass. At this stage, sugar solution (prepared separately) was added to the kettle and further heated with constant scraping. To this mix, powdered sugar was added and thoroughly mixed to get a dry mix. This mix packed in LDPE pouches has a shelf-life of about a year at ambient temperature. Palada payasam of required consistency could be prepared by cooking 200 g of the dry mix in half litre of toned milk for about 12 min.

Rai et al. (2002) also developed a method for the manufacture of dry palada payasam mix. In this method, ada flakes, ground sugar and skim milk powder were dry blended in 15: 60: 25 proportions, respectively. The sugar used in the mix contained 25% caramelized sugar which improved the flavour of the final product. Payasam could be prepared from the mix by cooking 44 g of the dry mix in 100 g of water for 10 min. The desired amount of fat (in the form of cream) was added during cooking.

**Avalakki payasam mix**

Avalakki i.e. beaten rice is a major ingredient in this payasam so the name Avalakki payasam is given. It is a popular delicacy in Karnataka and Kerala. Avalakki prepared by the traditional method has a low shelf-life i.e. less than 24 h at room temperature. Therefore, several attempts were made to develop a ready-to-reconstitute avalakki payasam dry mix.
According to a method, manufacture of ready-to-reconstitute *avalikki payasam* dry mix involves drying of soaked beaten rice along with milk and sugar. In another method, one fifth of the soaked beaten rice used for the final preparation along with milk and sugar (80 g) was concentrated in a steam kettle to a high viscous semisolid form. Sugar syrup and remaining wet flakes were added to the kettle and vigorously stirred to get the mix in the dry form (Rao, 2006).

**Gasa-gase payasam dry mix**

Nath et al. (2000) developed a process for production of dry mix of *gasa-gase payasam*, which has been commercialized. The process involved soaking of powdered *gasa-gase* and rice in water, mixing both of these ingredients along with coconut powder and grinding into a fine paste. The paste along with milk and sugar were concentrated in a stainless steel jacketed steam kettle and crystallized out as dry mix. Cardamom flavour was incorporated into the product at this stage. This product packed in polyethylene pouches kept well for more than 3 months at ambient temperature.

*Gasa-gase payasam* will be prepared by reconstitution of 300 g of the *gasa-gase* payasam dry mix into one litre of diluted milk (1:1 with water) followed by boiling for 2-3 min. This *payasam* compared well with the traditionally prepared product.

Rao et al. (2003) developed a modified method for the manufacture of *gasa-gase payasam* dry mix. This method consisted of roasting of rice (25 g), *gasa gase* (50 g), and grated *copra* (25 g) followed by dry grinding. The powdered ingredients were dry mixed with whole milk powder (40 g) and powdered sugar (200 g). The powdered sugar contained 12.5% of caramelized sugar in it. *Payasam* can be prepared from the dry mix by mixing 150 g of the mix in 250 ml toned milk diluted with equal quantity of water. The mixture was pressure cooked for a few seconds at 15 psi in a pressure cooker, and the cooked contents were then mixed for a few seconds in a home mixer for texture development.

**Ready-to-reconstitute fermented beverages**

**Ready-to-reconstitute pearl millet and sorghum lassi**

Mohan (2007) and Khetra (2008) developed a method for the manufacture of ready-to-reconstitute pearl millet and sorghum *lassi*, respectively. Milk solids in the form of concentrated skim milk and cream, and sorghum and pearl millet solids in form of dried malt were combined in a definite proportion; the mix was heated to 90°C for 10 min followed by cooling to 37°C. Later the mix was inoculated with starter culture viz. NCDC-167 and NCDC-263 followed by incubation at the same temperature for 10-12 h. The optimum levels of CSM and cereal solids were decided on the basis of convenience in spray drying, sensory evaluation and physicochemical properties of the reconstituted beverage. The fermented concentrated mass 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 was dry blended with spices and pectin to obtain Ready-to-reconstitute pearl millet or sorghum *lassi*.

**Developments in instant vermicelli kheer mix**

At DFRL, Mysore, Jayathilakan et al. (2000) developed a *kheer* mix based on reconditioned vermicelli, milk powder, sugar and flavourants. The product has been found to have the required chemical and microbiological stability for 6 months at 37°C. The product is regarded as highly suitable for all ages, particularly for hospital patients, school children and also useful for disaster relief operations.
Conclusions

A wide variety of traditional dairy delicacies, drawn from different regions of the country, are produced in India using processes such as heat and/or acid coagulation, desiccation and fermentation. Traditional milk sweets and dairy desserts form the large bulk of such products. However, tremendous economic potential of this sector has remained untapped because of the fact that the manufacture of traditional dairy products has remained confined to small level operations, which is manual and energy intensive. Therefore what is needed is adoption of existing unit operations and machines to upgrade and mechanize manufacturing processes of traditional dairy products. The products developed at NDRI, are an attempt in this direction. It is expected that such ready mixes would add convenience to the existing array of customary dishes. Value addition to this effect will not only help in exploiting domestic market-reach of our dairy products but also open new vistas for export to neighbouring countries.

References


Introduction

Bovine milk fat occupies the 6th position in the worldwide production of edible fats and oil (Hill, 2000). It represents a ubiquitous and important source of dietary fat which is mainly assimilated as butter, cream, ghee, butter oil and table spreads. Application of enzymes is in the chemical redesign of milk fat for improving physical, chemical and/or nutritional properties. Convenience, safety, nutritional balance and sensory satisfaction are the basic driving forces behind the modification of existing fat products and the development of new ones.

Controlled hydrolysis of milk fat for flavour enrichment in dairy and non dairy foods

The triacylglycerols of milk fat possess an unusually large proportion of short-chain fatty acid residues. Lipase-catalyzed release of these moieties as free fatty acids can impart sensations of richness, creaminess, buttery flavor and a variety of cheese aromas to the product. Controlled enzymatic hydrolysis of milk fat has been used in the dairy industry to produce butter-like or cheese-like flavor products and additives.

Lipolyzed milk fat has been extensively used in oils, fats, cereals, snacks and baked goods. One classic example is the oil used to pop corn, or to cover popped corn.

Manufacturing Process:

Lipolyzed milk fat emulsions, which may vary in fat content from 25 to 95% with an average of 50%, are the most usual commercial form of lipase-treated milk fat (Fig. 1). Arnold et al., (1975) have shown that butterfat, modified by lipases from different sources, improved the flavor of bread after 24 h of storage if 35-40% of the shortening was replaced by enzyme-modified butterfat. Recognition of the potential of milk fat in terms of production of flavors for use within the food industry has prompted numerous applications of lipase-catalyzed hydrolyses and several patents have been issued on enzyme-modified milk fat products and additives for use in baked products and other foods.
Fig. 1 Milk fat modification for flavour enrichment

Table: 1. Major commercial available natural dairy flavour ingredients

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Manufacturer</th>
<th>Description</th>
<th>Major application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marstar L-33</td>
<td>Miles Laboratories</td>
<td>Lipolyzed cultured milk or cream</td>
<td>Caramel candy</td>
</tr>
<tr>
<td>DAC-1600</td>
<td>Dairyland Food Laboratories</td>
<td>Lipolyzed cultured milk or cream</td>
<td>Caramel candy</td>
</tr>
<tr>
<td>Butter Buds</td>
<td>Morton-Norwich</td>
<td>Lipolyzed milkfat</td>
<td>Butterscotch hard Candy</td>
</tr>
<tr>
<td>MIL LAIT</td>
<td>Dairyland Food Laboratories</td>
<td>Modified whole milk powder</td>
<td></td>
</tr>
<tr>
<td>Dariteen L-22</td>
<td>Miles Laboratories</td>
<td>Lipolyzed cream flavor</td>
<td>Cheddar cheese soup, caramel candy</td>
</tr>
<tr>
<td>Dariteen L-95</td>
<td>Miles Laboratories</td>
<td>Lipolyzed butteroil</td>
<td>Butterscotch hard Candy</td>
</tr>
</tbody>
</table>

The lipolyzed cultured cream products were prepared by pretreating cream with lipases and then subject to thermal treatment prior to inoculation with *Lactobacillus bulgaricus* to develop further acidity (arising mainly from formation of lactic acid). These products have potential to enhance dairy flavors in candies, cheesecakes, sauces, dips, salad dressings, sweet doughs, soups and baked goods naturally and thereby restrict artificial flavours in the formulation. Typical rate of addition is 0.05-0.10% for subtle flavorings and 0.1-0.5% for more pronounced flavors, based on weight of finished product.

**Innovations in dairy spread manufacturing process**

Use of milk fat in spread has been restricted due to its poor spreadability at refrigerated temperature. To overcome this problem, various alternatives, such as fractionation, selective blending, and chemical or enzymatic processes to produce specialty milk fat ingredients have been applied. Among these processes, the enzymatic interesterification (EIE) is a promising technique for the production of structured lipids in recent time. Since blending does not result in the modification in the chemical properties of TAG composition, further modification is needed to modify the TAG composition which may resulting in the modification of physical behavior of the products. Interesterification (IE) is the process of re-arranging the fatty esters within and between triglycerides resulting in most cases, a change in the physical properties of the oil/fat. Interesterification is the intra and intermolecular exchange of fatty acids on the glycerol backbone of triacylglycerols, When compared to simple fat blending, enzymatic interesterification results in new products with different triacylglycerol (TAG) composition, and consequently modified physical properties.
Enzymatic processes are interesting because enzymes are recyclable, eco-friendly and non-toxic materials. Lipase-catalyzed interesterification also has advantages compared to the chemical process, such as mild conditions, fewer side products (diacylglycerols, monoacylglycerols, and free fatty acids) and reaction specificity (substrate and positional specificity and stereospecificity). Interesterification of milk fat has been carried out with immobilized lipases, in both solvent and solvent-free systems, in a batch-type reactor or a continuous packed-bed reactor, at temperatures from 37 to 80 °C.

Blends of milk fat and canola oil (MF:CNO) were enzymatically interesterified (EIE) by Rhizopus oryzae lipase immobilized on polysiloxane-polyvinyl alcohol (SiO$_2$-PVA) composite system. Effect of different proportions of blends of MF:CNO (50:50, 65:35 and 80:20) and temperatures (45, 55 and 65 °C) on the composition and texture properties of the intersterified products was assessed by measuring the interesterification degree (ID) and consistency (at 10 °C) as response variables. A blend with 65 % of milk fat and 35 % of canola oil, and temperature of 45 °C has adjudged has the best combination with 19.77% ID and the consistency at 10 °C was 56290 Pa. The potential of this eco-friendly process demonstrated that a product could be obtained with the desirable milk fat flavour and better spreadability under refrigerated conditions (Morais Nunes et al., 2011).

**Interesterification of milk fat**

Milk fat has appealing sensory attributes. It has high melting point TAG that limit its consumption and use, mainly due to its poor spreadability when refrigerated. Interesterification leads to exchange of fatty acids on the glycerol backbone or to change in the position of fatty acids on the glycerides. Interesterification leads to modifications in TAG composition and consequently, in its physical characteristics such as melting and crystallization behaviours. Rearrangement of fatty acids on their glycerol backbone offers the possibility of changing the physical characteristics, especially the crystallization properties of the fat product. The changing behaviour of physical characteristics in the interesterification process is due to the fact that most of the oil has the second position of the TAG molecules occupied by unsaturated fatty acids and the interesterification process will result in random distribution of fatty acids. The rearrangement process does not change the degree of unsaturation or the isomeric state of the FA as they shift from one position to another. The stability of the oils and fats also remains essentially unchanged. Therefore, interesterification between two or more fats or oils obtain in a completely different TAG composition compare to the starting materials.

**Development of human milk fat substitutes (HMFS) using milk fat**

Although human milk is the first choice for newborn infants, milk substitutes play an indispensable role in infant nutrition when breastfeeding is not possible, desirable or sufficient.
Generally, the fat in infant formulas mainly comes from cow milk fat or vegetable oils in which the composition and structure of fatty acids are significantly different from those in human milk. Fig. 2 reveals the differences of the fat in human milk and cow milk. In cow milk fat, the content of low-carbon chain saturated fatty acids (C10-C16) is higher while the quantities of polyunsaturated fatty acids such as C18 : 2, C18 : 3 and C20 : 4 are lower than those in human milk. In addition, the saturated fatty acids in cow milk are mainly esterified at sn-1 and 3 positions, whereas those for breast milk (mainly C16 : 0) are esterified at sn-2 position. Carnielli et al., (1995, 1996) studied the effects of the palmitic acids in different stereoisomeric positions on preterm and full-term infants, and they observed a significantly higher absorption of fatty acids and calcium in infants fed formulas containing triglycerides similar to those found in human milk (palmitic acids were esterified predominantly at sn-2 position) compared with infants fed regular formulas (with the palmitic acid esterified mainly at sn-1 and 3 positions).

Human milk fat substitutes can be produced by enzymatic interesterification reaction. Christensen and Homer (1993) prepared a HMFS through Rhizo-mucormiehei lipase-catalyzed modification of butteroil.

Ann-Dorit et al., (2010) combined butterfat with soybean oil and rapeseed oil, then the raw materials were catalyzed with Lipozyme RM IM to produce HMFS (46% of fatty acids were esterified at sn-2 position of triacylglycerols) with a molecular structure and fatty acid composition which were similar to those in human milk fat. The oxidative stability of HMFS was lower than that of the reference oil with the same fatty acid composition.

**Development emulsifiers from milk fat**

Monoacylglycerols (MAG) and diacylglycerols (DAG) are widely used as emulsifiers in food systems, where they account for 75% of the world production of emulsifiers, as well as in the pharmaceutical and cosmetic industries. MAG and DAG can be formed by controlled hydrolysis of triacylglycerols or controlled esterification of glycerol and fatty acids, or alternatively, via acyl exchange between excess glycerol and triacylglycerols (an alcoholysis process frequently termed glycerolysis). Yang et al., (1994) have screened several commercial lipase preparations (porcine pancreatic lipase, Candida cylindracea, Rhizopus arrhizus, Pseudomonas spp., Rhizopus javanicus, Rhizopus delemar, Geotrichum candidum and Mucor javanicus) for alcoholysis of
milkfat in the presence and absence of solvent with the purpose of producing MAG and DAG from milk fat.

**Reduction of cholesterol level in milk fat**

Milk fat contains, on average, 7.3 mg cholesterol per gram of fat. Enzyme cholesterol reductase was used to convert cholesterol to coprostanol as later is poorly absorbed in our digestive tract. *Lactobacillus acidophilus* ATCC 314, *L. acidophilus* FTCC 0291, *Lactobacillus bulgaricus* FTCC 0411, *L. bulgaricus* FTDC 1311, and *L. casei* ATCC 393 showed greater hydrophobicity properties. These were able reduce cholesterol via conversion of cholesterol to coprostanol, by producing cholesterol reductase (Lye et al., 2010). Other commercial method to reduce cholesterol includes, addition of beta cyclodextrin. A study was carried out by (Alonso et al., 2009) to determine optimum conditions (β-cyclodextrin concentration, mixing time, and holding time) for cholesterol removal from pasteurized non-homogenized milk. The β-cyclodextrin (0.4, 0.6, 0.8, and 1.0%) helped to remove 65.42 to 95.31% of cholesterol at 4 °C in 20 min. The β-cyclodextrin-cholesterol complex was precipitated from milk during 20 min without stirring at 4 °C and removed by centrifugation. After separating the milk, approximately 0.35% of residual β-cyclodextrin was remained in the skim fraction and 0.1% in the cream fraction, when milk treated with 0.6% beta-cyclodextrin (optimum level). The rest of the β-cyclodextrin was complexed with the cholesterol and eliminated via the discharger of the separator. Individual fatty acid and triglyceride compositions did not differ between control milk and milk treated with 0.6% β-cyclodextrin.

**Herbal Ghee:** Arjuna, a traditional Indian herb, is used widely for heart related benefits. It regularizes the heartbeats, strengthens heart muscles, reduces bleeding and inflammation. Commercially viable technology for manufacturing Herbal Ghee incorporating active components of Arjuna was developed at NDRI. The developed ghee was found sensorily similar to the market ghee. It had overall acceptability score of 85.1 compared to the control (90.8). It complies to all the conditions laid down by FSSAI and AgMark (BR Reading 42, Moisture 0.13%, FFA, 0.36%, RM value 28.2). The Arjuna ghee was found to be highly stable (8 days at 80 °C) as compared to control ghee (2 days at 80 °C as noted in accelerated storage studies). This is due to the fact that Arjuna extract contains several antioxidants like polyphenols and terpenoids in addition to phytosterol which are also beneficial in conditions of cardiovascular diseases (CVD) and blood pressure. Thus, Arjuna ghee can be consumed instead of normal ghee in order to reduce the risk of CVD and to boost up the immune system.

**References:**


Application of Artificial Neural Network (ANN) in Food Processing and Sensory Evaluation

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Introduction

Artificial neural networks (ANNs) have been successfully applied in the area of food science over the past two decades. However, most applications are in the development stage. ANNs are useful tools for food safety and quality analyses, which include modeling of microbial growth and from this predicting food safety, interpreting spectroscopic data, and predicting physical, chemical, functional and sensory properties of various food products during storage, processing and distribution. ANNs is a promising tool for modeling complex tasks in sensory evaluation, process control and simulation, machine vision and electronic nose for food safety and quality control.

Artificial neural network refers to an information processing system (paradigm) that simulates functioning of biological nervous system, such as the brain, for processing of information (MacKay, 2005). Neuron is the basic structural element and information processing module of the brain. A typical human brain has an enormous number of neurons arranged in a highly complex, nonlinear, and parallel structure. As a result, the human brain has a very efficient structure for information processing, learning and reasoning. An artificial neural network (ANN) is composed of a large number of highly interconnected artificial neurons (processing elements) that uses a mathematical or computational model for information processing based on a connectionist approach to computation. Like human beings, ANNs learn by example. Learning in biological systems involves adjustments to the synaptic connections that exist between neurons. This is true of ANNs as well. ANN is an adaptive system that changes and adjusts its structure based on external or internal information that flows through the network.

Neural networks have remarkable ability to derive meaning from a large pool of data which could be complicated and imprecise. It has the ability to recognize patterns and detect trends that are too complex to be noticed either by humans or even other computer techniques. The network, when trained with certain set of data, becomes an expert to analyze the pattern and information, it has been provided. Whenever new situations of interest emerge, it can thus draw inference based on its previous experience with the dataset.

Basic model of a neuron

The basic idea of neural networks is to simulate the functioning of the human brain that has a basic unit called a neuron (Fig. 1). A simplified biological neuron consists of four parts: dendrites, soma, axon, and synapses. Dendrites are chemical receptors that receive signals from other neurons. The soma is the cell body of a neuron that processes the input signals. An axon is a chemical emitter that sends out the processed signals to nearby neurons. Synapses are the junctions connecting neurons and regulate the signal transmittance between neurons. Although the structure and function of actual biological neurons is much more complicated, this simplified biological neuron serves as the basis of ANNs.
Similarly in ANN, the neuron (processing element) is a fundamental unit or building block of the ANN (Kartalopoulos, 2003). The neuron sums the weighed inputs and passes the result to a transfer function to generate an output. The output information is then sent to another neuron as input or is used directly as a network result. The weights are connection strengths between neurons. Because some input signals may be more important than others, the use of weights corresponding to the importance of each input signal provides an efficient way to generate ideal outputs. Weights are adjustable during network training and there are various algorithms to adjust the weights during network training.

Processing inside the basic element (neuron) is shown in figure 2 given below. A neuron has a set of n inputs $X_j$ where $J$ varies from 1 to $n$ and indicates the source of input signal. Each input $X_j$ is weighted before reaching the main body of the processing element by the connection strength or the weight factor $W_j$ ($X_j$ is multiplied by $W_j$). In addition, it has a biased term $b_0$, a threshold value $\theta$ that has to be reached or exceeded for the neuron to produce a signal, a nonlinearity function $F$ that acts on the produced signal, and an output $O$ after the nonlinearity function. When the neuron is part of network of many neurons, it is referred to as a node. For $m$ nodes in the network, inputs, weights, activation signals, output, threshold, and nonlinear functions are termed as $X_{ij}$, $W_{ij}$, $R_i$, $O_i$, $\theta_i$, $F_i$ respectively. The basic model of a neuron is illustrated in the following figure 2.

$$O_i = F_i \left( \sum_{j=1}^{n} W_{ij} X_j \right)$$

(1)

The neurons firing condition is
\[ \sum_{j=1}^{n} W_{ij} X_j > \theta \]  

Where, \( i \) represents the neurons and \( j \) represents the input source.

The purpose of the nonlinearity function is to ensure that the neurons response is bound. Some frequently used functions are log sigmoid, hyperbolic, hard limit and Gaussian functions.

\[
\text{Log Sigmoid Function, } f(x) = \frac{1}{1+e^{-x}} \text{ or } \frac{e^x}{1+e^x} 
\]

\[
\text{Hyperbolic tan Sigmoid Function, } f(x) = \frac{2}{1+e^{-2x}} - 1
\]

\[
\text{Hard Limit Function, } f(x) = \begin{cases} 1, & \text{if } x > 0; 0 \text{ Otherwise} \end{cases} \text{ or } f(x) = \begin{cases} 1, & \text{if } x >= 0; -1 \text{ Otherwise.} \end{cases}
\]

Neurons form layers with different types of connections among neurons (as shown in Figure 3). A neuron of one layer is always connected with neurons of at least one other layer. There are different types of connections between neurons. For inter-layer connections, a neuron in one layer is connected to all the neurons in the next layer, producing a fully connected network; if the neurons are connected to only some of the neurons in the next layer then the network is partially connected. Neurons in one layer send output information to the next layer.

**Learning in ANN**

The most challenging assignment in developing an ANN is to make the network learn a given task. Two important learning techniques that are frequently used are delineated below.

1. **Supervised Learning:** In this learning mode, input and output targets are fed to the neural network. The network computes the output based on the inputs and then tries to minimize the error difference between the actual target output and computed output by adjusting the weights and biases of input source in an iterative manner.

2. **Un-Supervised Learning:** In contrast to supervised learning it does not require target output. During the training session, the neural network receives many input patterns and it arbitrarily organizes the patterns into categories. When a new input is given at a later stage, the neural net provides an output response indicating the class to which the input belongs. If a class is not found for the new input, a new class is generated.

**Network topology or architecture**

Topology refers to the interconnection of neurons and their layout in a neural network. It also describes the flow of signals from one layer to another. Two important topologies used in ANN are feed-forward and feed-back networks.

- **Feed-forward network:** Feed-forward ANNs allow signals to travel one way only i.e., from input to output. There is no feed back (loops) i.e., the output of any layer does not affect the same layer. These networks are straight forward networks that associate inputs with outputs. These are used for prediction and pattern recognition. This type of organization is also referred to as bottom-up or top-down.
- **Feed-back network:** Feed-back networks can have signals traveling in both directions by introducing loops in the networks. These networks are very powerful but can be extremely complicated. Feed-back networks are dynamic, their state is changing continuously until they reach an equilibrium point. Feed-back topology is also referred to as interactive or recurrent.

**Network Layers**

One of the most commonly used models is a three-layer feedforward network. In this type of network (Figure 3), the input layer receives input information from an input file or from electrical sensors in an on-line application, and passes this information to the next layer of neurons. The input layer normally does not conduct signal processing. The third layer, the output layer, processes the input information from the previous layer and then transfers information out from the network. Layers between the input and output layer are called hidden layers. Generally one hidden layer is sufficient for a network, although more than one hidden layer may be used for complicated applications.

![Figure 3. Fully connected neuron layers.](image)

**Input Layer:** Input layer represents the raw information that is fed into the network. The number of neurons in input layer depends on the input parameters.

**Hidden Layer:** The activity of hidden layer is determined by the input layer and the weights on the connections between the input and the hidden layer. Hidden layer temporarily stores the processed data in between the input and output layers.

**Output Layer:** Output layer produces the final response of the network and behavior of the output layer depends on the activity of hidden layer and the weights between the hidden and output layer.

In a network there may be one or more hidden layer that depends on the complexity of the input data.

In general, two hidden layers are sufficient for solving dairy applications. The number of neurons in hidden layer also depends on the complexity of data. There is no general method to predict the required number of layers and number of neurons in each layer to get best results. It has to be determined through iterative process by changing the permutation and combination of different set of values of hidden layers and number of neurons in respective hidden layers.

Over the period, several ANN models have been developed to represent the biological neural network in best way. Few such important network models are Perceptron (single layer and multi layer) model; ADALINE and MADALINE model; Feed-forward with back propagation; Hopfield model; Competitive learning model; Real time models; Probabilistic neural network model; Radial basis function model; Kohnnen self organization model etc.
Network Performance Parameters

The performance of designed network is evaluated based on some performance parameters. A few performance parameters commonly used are as follows.

1. Sum of squares of error (SSE) = \( \sum (O_i - E_i)^2 \)

2. Mean sum of square of errors (MSE) = \( \frac{1}{n} \sum (O_i - E_i)^2 \)

3. Percent root mean square (% RMS) = \( \frac{100}{\sqrt{\frac{1}{n} \sum \left( \frac{O_i - E_i}{O_i} \right)^2}} \times 100 \)

Where, \( O_i = \text{Observed value and } E_i = \text{Expected value} \)

Feedforward Neural Networks with Backpropagation Algorithm

Backpropagation (BP) is the most commonly used algorithm to train feed forward neural networks. The BP algorithm is normally used to train neural networks that have only inter-layer connections and no intra-layer connections. In a BP trained neural network, information from the input layer is feedforwarded through zero or more hidden layer to the output layer. The output errors are back-propagated from the output layer through the hidden layer(s) to the input layer. Error term for each output layer node is computed as given below (equation 4).

\[ \delta_i = O_i(1 - O_i)(Y_i - O_i) \] (4)

These errors are used to adjust the connection weights between the last but one layer of the network and the output layer. New value of the weight \( W_{ji} \) of the connection from node \( j \) to node \( i \) is given by (equation 5):

\[ w_{ji}^{new} = w_{ji}^{old} + \eta O_j \delta_i \] (5)

Here \( \eta \) is known as learning rate and its value is chosen by trial and error by repeated runs on the training data. Typically the value of \( \eta \) ranges between 0.1 - 0.9. Low value give slow but steady learning whereas high value gives erratic learning and may lead to an unstable network.

The process is repeated for the connections between nodes in the last hidden layer and the last but one hidden layer. The backward propagation of weight adjustments like this continues until the input layer is reached. At this stage a new set of weights are computed and can be used in the next pass when presented with a training data observations. A single scan of all cases in the training data is called an epoch. Most applications of feedforward networks and backprop require several epochs before errors are reasonably small. A weakness of the neural network is that it can be easily overfitted, causing the error rate on validation data to be much larger than the error rate on the training data.

Important ANN parameters

While designing an ANN, one should consider the following parameters for getting optimum performance.

- Network topology or architecture
- Number of layers in the network
- Number of neurons or nodes per layer
- Learning algorithms to be adopted (in supervised case only)
- Number of iterations per patterns during training
- Network performance

The selection network architecture i.e. number of layers, neurons in each layer, epochs, training algorithms learning rate etc. is the most time consuming and complex task to train a neural network. The usual procedure is to make intelligent guesses using past experience and to do several trial and error runs on different architectures. Research continues on such methods. However, as of now there seems to be no automatic method that is clearly superior to the trial and error approach.

**Application of ANNs in food processing**

ANN has emerged as a very useful tool for optimizing processing parameters of a number of food processing operations. It is also being increasingly used for pattern recognition aimed at characterization of food properties and for predicting quality deterioration during storage life of the products. ANNs have been applied in almost every aspect of food science and technology during the past decade. Some of these applications areas are as follows:

- Machine perception including machine vision and the electronic nose
- Spectral data interpretation for identification of functional groups and quantitative analysis
- Food Microbiology and Food Fermentation
- Predicting physical, chemical, and functional properties of food products during processing and distribution.
- Prediction of shelf life of diary products based on sensory evaluation

**Machine Perception**

Machine perception is one of the most promising application areas of ANNs in the field of food science with the most significant being machine vision and electronic nose, which have brought a revolution in sensory analysis. Important sensory parameters such as the odor and appearance (including shape and color) of a food product can be detected by machine perception technologies, which can provide indications of overall food safety and quality.

**Electronic Noses**

Due to the vital impact of flavor and aroma in food products, enormous efforts are made to evaluate these factors in both the research community and in the food industry. Yet, because of the complex nature of food that involves interactions among a mixture of numerous components within the food, as well as the complex and disparate reaction of individual humans to the food, quantitative measures of food flavors and aroma can be difficult. Electronic nose is a fast screening tool to enhance objective evaluation of sensory quality of foods much superior to subjective human sensory panel results (Marsili, 1995). A response pattern is generated and then processed by a pattern recognition system to get objective information about the sample. Conventional methods for pattern recognition are generally linear including principal components analysis and partial least square regression. However, most of the time, the response patterns by sensors are nonlinear and linear methods may not provide a robust model for pattern recognition. The ANN technologies have been increasingly used as pattern recognition systems for electronic noses over the past decade. Some studies indicated that ANNs may be more adaptive and may be superior to conventional linear methods for pattern recognition (Eklov et al., 1998; Sinesio et al., 2000; Brezmes et al., 2001). Electronic nose systems have been applied in quality analysis and classification of various food systems including beverages, fruit, oil, grain, fish, meat, and dairy products, which normally have a relatively strong smell.
**Machine Vision**

Similar to electronic noses, the applications of machine vision in the food science area are to accomplish tasks related to visual quality control and/or remote sensing to replace human inspectors in an adverse environment, reduce inspection errors and/or increase efficiency. An example of how machine vision is applied to food analysis is provided in the work of Ding and Gunasekaran (1994). First, digital images of damaged and undamaged corn kernels, almonds and animal crackers (n = 144 with 72 damaged and 72 undamaged for each category) were acquired. Image features were then extracted using a statistical model-based feature extraction method (SMB) and a multi-index active model-based feature extraction method (MAM). For both methods, an average reference shape for undamaged objects was obtained. Then the shape of the inspected object was compared with the reference shape. The MAM method was more selective since the position, orientation and scale of an inspected object were adjusted to best fit the reference shape. In the subsequent pattern recognition, information about the differences between the average reference shape and shape of the inspected object was used as an input vector for an ANN and several other methods. The results showed that with an ANN for pattern recognition up to 98% classification accuracy was achieved

**Spectroscopic Data Interpretation**

The use of neural networks for spectral data interpretation includes both identification of functional groups and quantitative analysis. Spectral data may include infrared, mass, and ultrasonic spectra. Similar to other application areas, the feedforward ANN trained by BP is the most commonly used neural network for spectral data interpretation. During the network construction, spectral information, measurement of absorption intensity or compressed spectral information is used as an input vector, and the analyte concentration, or a desired physical or chemical characteristic is used as an output vector.

Park et al. (1994) used an ANN to predict sensory characteristics of beef from 72 beef carcasses from ultrasonic spectral data. Seven ultrasonic spectral features, including lower, upper, central and peak frequency, bandwidth, skewness and the number of local maxima were used as input information for the network. Sensory characteristics were scored on an eight point scale by seven trained panelists. The score of sensory characteristics were used as output neurons. The root mean square error of prediction (RMSEP) was in the range of 0.102–0.135 for the selected sensory features. ANN models outperformed other statistical regression models.

**Food Microbiology and Food Fermentation**

A commonly used technique for microbial growth modeling is response surface models developed through linear regression methods. Most recently, ANNs were used as an alternative method for modeling microbial growth. As in other fields, a majority of publications claim that ANN models achieve better agreement with experimental data than response surface models (Hajmeer et al., 1997; Lou and Nakai., 2001; Garca-Gimeno et al., 2002). Modeling of microbial growth includes directly predicting the number of microbes or the microbe growth and indirectly predicting the parameters of an existing model (Hajmeer et al., 1997; Garca-Gimeno et al., 2002). In addition to modeling microbial growth, ANNs are also applied for monitoring quality parameters during food fermentation, determining quality characteristics of fermented foods, classifying fermented products, and classifying defect foods due to microbial contamination. Several applications in fermentation have been developed using feedforward neural network models. In one study, Syu et al. (1994) applied ANNs to predict ethanol concentration during a brewing process. The initial free amino concentration, oxygen concentration, and viable cell count were used as input vector for the network construction.
Ethanol concentrations at seven different periods during the fermentation were used as the output vector. The predicted results by the ANN model were close to experimental data.

**Other Applications**

Mittal and Zhang (2000; 2001) developed artificial neural networks to predict heat and mass transfer during deep fat frying of slab shaped foods with edible coatings and meat balls during deep fat frying. Frying time, slab half thickness / radius of meat balls, film thickness, food initial temperature, oil temperature, moisture diffusivity, fat diffusivity, thermal diffusivity, heat transfer coefficients, and fat conductivity were the input variables whereas temperature at the centre, average temperature, fat content and average moisture contents were the outputs.

Drying processes have been successfully modeled by several workers in the past. Calculations concerning the prediction of moisture content and temperature of the dried product could be done by Kaminski and Tomczak (2000) by keeping the air flow rate and drying agent temperature as the input parameters. Islam et al., (2003) developed ANN based liquid diffusion model for potato slices of different thickness using air at different velocities, humidities and temperatures. Moisture content and temperature dependence of the liquid diffusivity as well as the heat of wetting for bound moisture were included in the diffusion model making it a highly non linear system. Zhang et al., (2002) developed ANN for rough rice drying to predict six performance indices namely energy consumption, kernel cracking, final moisture content, moisture removal rate, drying intensity and water mass removal rate. Four drying parameters viz. rice layer thickness, hot air flow rate, hot air temperature and drying time were the inputs of the neural network.

Pattern recognition and classification are the areas where ANNs have been found to deliver excellent results. Quantification of morphological, colour and textural properties for classification of agricultural products have been significantly improved due to application of ANN tools while using computer vision-classifiers (Jayas et al., 2000; Patiwal et al., 2003).

ANN has been successfully applied in the past for predicting beef sensory quality (Park et al.,1994), shelf life of pasteurized milk (Vallejo-Cordoba et al., 1995), sensory attributes of noodles (Tulbek et al., 2003) and shelf life of soya milk (Ko et al., 2000).

Food extrusion process modeling is a very difficult task due to numerous complexities. As a food extruder is a multiple input and multiple output (MIMO) system, dynamic changes in torque, specific mechanical energies, and pressure were modeled and controlled using two independently trained feed forward artificial neural networks by Eerikainen et al., (1994). Ganzyal et al., (2006) developed ANNs to predict expansion ratios, water absorption index, and water solubility index individually from moisture content, screw speed, and barrel temperature while extruding rice flour and rice starch through a simple screw extruder. Ganzyal et al., (2003) also developed neural networks (NN) models for selected properties of waxy maize cross-linked starches, extruded at different levels of moisture contents and different screw speeds. The predicted properties were expansion ratio, unit density, bulk density, water absorption index and water solubility index.

**ANN work at NDRI**

In a laboratory at NDRI, a multilayer feed forward neural network model with back propagation algorithm was used for predicting shelf life of UHT milk employing MATLAB software. Bayesian regularization algorithm provided the most stable and consistent results for the given data set. The input parameters were: reflectance (instrumental measurement of colour), total hydroxy methyl furfural (measure of browning), thiobarbituric acid (measure of oxidative rancidity), free fatty acid (measure of lipolytic spoilage) and trinitro benzene sulphonic acid (indicative of proteolytic
degradation). The output parameters were sensorily evaluated for flavour and total sensory score, which represented storage life of the product. Two network components namely number of layers (1 & 2) and number of neurons in each layer (3 to 25) were used. The transformation function on each hidden layer was tangent sigmoid while that transformation function on the output layer was linear. The perceivably lower %RMS error (5.85 for flavour and 4.20 for total sensory score) indicated that the developed ANN models gave excellent accuracy of prediction. The ANN approach was also tried for predicting sensory quality of the Basundi mix. On account of their higher correlation coefficients pH, HMF, bulk density and insolubility index were used as the input parameters. The other input parameters were temperature and storage period. The two output parameters were again the flavour and the overall acceptability score. In this case too, the Bayesian regularization algorithm provided the most stable and consistent results. Two network components namely number of layers (1 & 2) and number of neurons in each layer (3 to 25) were used. Again a relatively low magnitude of percent root mean square error (%RMS 3.72 for flavour and 1.85 for total sensory score) indicated an excellent fit.

**References:**


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Application of SSHE for Mechanized Production of Indian Dairy Products

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Introduction
Indian milk sweets have played a significant role in the economic, social, religious and 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 led India to emerge as the largest milk producer in the world. It is estimated that milk production in India reached a record level of 127.0 million in the year 2012 accounting for more than 17% of the world’s total production and about 50-55% of milk produced is converted by the traditional sector (halwai) into variety of Indian milk products. The market of Indian milk products 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 these 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 reasons 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 sweets derived therefrom should be taken up by organized plants. The commission also suggested that efforts 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 packaging with minimum expenses. For handling high viscosity products with or without particles, and for the products that tends to foul the heat transfer surface, the scraped surface heat exchanger (SSHE) is most suitable. In SSHE, the working fluid is spread in the form of a film over the heat transfer surface 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 which 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 rabri.

Two stage scraped surface heat exchanger (SSHE)
Two stage SSHE was conceived for continuous manufacture of khoa and the system was arranged in cascade fashion. The rotor of first SSHE was provided with four variable clearance blades and operated at 3.3 rotations per second (rps). Milk would concentrate in it upto 40-45% total solids in the first SSHE. This concentrate enters the second SSHE which has two variable clearance blades and two helical blades operating at a lower rpm of 2.5 rps. The steam pressure is adjusted to 250 kPa in the jackets of both the SSHE and milk flow rate is kept constant at 150 kg/h. Khoa is collected from the outlet of the second heat exchanger. khoa made from two stage TFSSHE is similar to that made from batch process. In this unit there was a saving of 66 kg steam for every 100 kg khoa compared to conventional method using steam jacketed kettle.

Three stage scraped surface heat exchanger (SSHE)
Based on the work done earlier with two stage SSHE, the design of three stage SSHE was conceived. It consists of three identical thin film scraped surface heat exchangers. 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. The steam inlets are provided with Pressure gauges, I/P converter, Transmitter, pneumatic valves, air pressure indicator and process controllers for automatic steam controlled system.

**Continuous khoa making**

Khoa is partially dehydrated, heat desiccated milk product and is widely used as a base material for preparation of numerous indigenous sweets. The increased demand of khoa based sweets has created need for large scale production ensuring uniform product quality, product safety, energy conservation etc. In this direction three stage SSHE with state of the art technology by incorporating various operating features and instrumentation. The complete system and its working are described elsewhere (Nanadkishore, 2010). The performance of the system was evaluated for continuous manufacture of khoa.

Standardized buffalo milk (2, 3, 4 and 6% fat) was obtained by adding required amount of skim milk, separated from raw buffalo milk into the balance tank. Khoa was manufactured by using 3-stage thin film scraped surface heat exchanger. Following process parameters were selected based on the experience gained through running of 2-stage SSHE. The rotor speed of first and second stage was kept at 200 rpm and 3-stage was varied as 20, 30 and 40 rpm. The steam pressure was kept as 4 and 2 kg/cm² in first and second stage and varied between 1 to 1.5 kg/cm² in third stage in such a way as to get final product of nearly uniform consistency. The flow rate of milk in all trials was kept fixed as 200 kg/h.

Table 1 gives the sensory evaluation score of the product obtained by variation in speed of third stage SSHE for different fat levels.

The data given in table indicate that keeping the rotor speed of third stage SSHE at 20 rpm, the sensory scores of all samples were better compared to 30 and 40 rpm. It is also seen that khoa made from continuous process was compared with traditional method.

**Continuous basundi making**

*Burundi* is a traditional heat desiccated milk delicacy having sweetish caramel and pleasant aroma, light to medium brown colour, thick body and creamy consistency with or without soft textured flakes that are uniformly suspended throughout the product. It contains all the solids of milk in an appropriate concentration plus additional sugar and dry fruits. It is consumed directly as a delicious sweet dish. It is most popular in Maharashtra, Gujarat and parts of Karnataka and is mainly prepared at home by the housewives on some special occasions like festivals, weddings etc. and relished due to its rich, caramel, pleasant and nutty flavor and thick consistency (Pagote, 2003).

Total annual production of *basundi* during 1996 was estimated to be 25000 tones and was mainly confined to cottage scale in non-organized sector (Aneja, 1997). Now-a-days, the popularity and demand of *basundi* is increasing due to its delicacy. Hence its production and marketing is increasing in a few big cities of the country.

**Preparation of basundi 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 SSHEs, which were located at the rear side of three stages SSHE, are opened manually. The feed pump is then started and flow is varied between 100-200 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 and second SSHE is switched on and the speed of both SSHE’s are kept between 100 to 175 rpm. Milk is first concentrated in first stage SSHE and then enters into the second stage where it is further concentrated. The mass flow rate is approximately so adjusted to get the concentration required in the basundi. From second stage TFSSHE, the product formed is collected and cooled to 10°C and then dry fruits were added to it @ 1.5% w/w of the basundi.
Combinations of scraper blade speed with different types of sugar were undertaken to get the best possible basundi out of SSHE. The result obtained while performing statistical analysis based on the sensory score, proximate composition analysis and physico-chemical analysis concluded that the best possible combination for preparing basundi was made by keeping rpm of first and second stage as 125 and steam pressure as 1.5 kg/cm² in both SSHE and using caramelizes sugar gave product of excellent quality.

The basundi prepared by best possible combination of operating parameters in SSHE has been compared with the basundi prepared by standard batch process in proximate composition and physico-chemical characteristics and found that both types of basundi were almost similar.

**Continuous rabri making**

First the buffalo milk is taken, filtered and standardized to a 6% fat and 9% SNF. The milk is 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 0.005 unit 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 stages 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 and second SSHE is switched on and the scraper speed of first and second SSHE are kept between 100 to 150 rpm (1.968 – 2.953 m/s) whereas third SSHE is kept at 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 formation 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°C and packaged in cups then dry fruits were added to it and rabri cup is finally wrapped with aluminium foil. The product was then stored at temperature lower than 10°C.

The rabri prepared by using 3% sugar 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. 1st and 2nd scraper speed have the significant effect on the sensory attributes, FFA, HMF, colour values (L*, a*, b*), firmness, stickiness of product, *rabri*.

**Continuous burfi making**

*Burfi* is the most popular *Khoa* based traditional confection all over India. The generic nomenclature “*Burfi*” covers a wide range of product variations that include plain, danedar, dudh, chocolate, fruit and coconut *Burfi*. It has variation in flavor, color, body and texture. *Burfi* is a popular milk based sweet in which the base material is essentially *khoa*. Sugar is added in different proportions and other ingredients incorporated according to the demand of consumers. *Burfi* is prepared by heating a mixture of milk solids (*khou*) and sugar to a homogenous consistency followed by cooling and cutting into small cubes. Even today, regardless of volume of production, *Burfi* is manufactured primarily in jacketed kettles by *‘halwais’*, which inherently suffers from several disadvantages such as low heat transfer rates, high fouling behavior, batch to batch variation in product quality, poor hygiene and sanitary conditions.

In order to manufacture *burfi* continuously the buffalo milk was taken, filtered and standardized to a fat 6.0% and SNF 9.0%. This standardized milk is to be preheated 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 stages 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 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, 2 kg/cm² and 1.5 to 2 kg/cm² respectively. Milk is first concentrated in first stage SSHE and then enters into the second stage where it is further concentrated. In third stage, we adjust the steam pressure between 1.5 kg/cm² to 2.0 kg/cm² accordingly by observing the body of 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 into pieces.

**Conclusion**

Three stage SSHE which has been developed with state of the art technology by incorporating various instrumentation and process controllers has proved very successful for large production of Indian milk products.

**References**


Fresh Buffalo Milk

\[ \downarrow \]

Filtration

\[ \downarrow \]

Standardization

(Fat = 6.0%; SNF = 9.0%; Acidity 0.17% LA)

\[ \downarrow \]

Sugar (5% on the milk basis), either white crystalline sugar or caramelized sugar syrup solution

Balance Tank

\[ \downarrow \]

Concentration in TFSSHE

\[ \downarrow \]

Collection of compact mass in well greased plates

\[ \downarrow \]

Cooling at room temperature

\[ \downarrow \]

Spreading into uniform thick layer

\[ \downarrow \]

Covering with aluminum foil
Fig.1 Schematic Diagram of Experimental Set-Up
Introduction

Color is one of the major attributes which affect the consumer perception of quality. In foods, the appearance is a primary criterion in making purchasing decisions (Kays, 1991). Color is very important for dairy products like traditional Indian dairy sweets, ice-creams etc. Appearance is utilized throughout the production-storage-marketing-utilization chain as the primary means of judging the quality of individual units of product (Kays, 1999). There are many other reasons for its importance, chief among them being standardization of the product (the consumer is suspicious of the same brand having widely variable colors); utilization as a measure of quality and economic worth; and utilization as an indicator of biological and/or physicochemical breakdown, and as a predictor of other quality characteristics (Taub and Singh, 2010). Color may be defined as the impact of the wavelengths of light in the visual spectrum from 390 to 760 nm on the human retina. The cells in the retina send a signal via the optic nerve to the brain which in turn interprets the responses in terms of what we call color (Francis, 1995). Human eyes can only perform qualitative color analysis. Therefore instruments are used for color quantification. There are various scales/models by which color can be numerically expressed.

Color Models

Munsell color scale

Albert H. Munsell devised a color ordering system based on human perception. A great number of paper color chips of different hue (Munsell Hue), lightness (Munsell Value), and saturation (Munsell Chroma) were used for visual comparison with a specimen color. In this system, any given color is expressed as a letter/number combination (H V/C) in terms of its hue (H), value (V), and chroma (C) as visually evaluated using the Munsell Color Charts (Geotek, 2012). For example, for the color with H=6.0R, V=3.0, and C=12.0, the Munsell notation would be: 6.OR 3.0/12.0. Hue, lightness or value and chroma is defined by ASTM (1989) as:

Hue: The attribute of color perception by means of which a color is judged to be red, orange, yellow, green, blue, purple, or intermediate between adjacent pairs of these, considered in a closed ring (red and purple being an adjacent pair).

Lightness: is the attribute of color perception by which a non-self-luminous body is judged to reflect more or less light.

Munsell value: is an attribute of color used in the Munsell color system to indicate the lightness of a specimen viewed in daylight, on a scale extending from 0 for ideal black to 10 for ideal white, in steps that are visually approximately equal in magnitude.

Chroma: The attribute of color used to indicate the degree of departure of the color from a gray of the same lightness.
**Munsell chroma:** An attribute of color used in the Munsell color system to indicate the degree of departure of a color from a gray of the same Munsell value, in steps that are visually approximately equal in magnitude.

**CIE XYZ**

The CIE developed the “XYZ color system”, also known as the “standard color system.” It is still used as a standard reference for defining colors perceived by the human eye, and as a reference for other color spaces. Like the RGB color model with additive primaries, CIE-XYZ uses 3 spectrally defined imaginary primaries: X, Y, and Z which are the representation of color (electromagnetic waves) that may be combined to describe all colors visible to the standard observer (LaCie, 2006).

**CIE xyY**

In order to effectively represent a three-dimensional figure on a two-dimensional sheet of paper, the CIE transformed the three-dimensional color space into two artificial dimensions of color (collectively called chromaticity) and one of intensity. A two-dimensional slice through this space was taken at the level of maximum intensity. This slice became the chromaticity diagram also called the CIE xyY Chromaticity Diagram (LaCie, 2006).

**Hunter L*a*b***

The Hunter Lab color scale evolved during the 1950s and 1960s. The Hunter Lab color scale is more visually uniform than the XYZ color scale. In a uniform color scale, the differences between points plotted in the color space correspond to visual differences between the colors plotted. The Hunter Lab color space is organized in cube form. The L axis is from top to bottom.
When a color is expressed in CIE L*a* b*, L* defines lightness, a* denotes the red/green value and b* the yellow/blue value. The CIE L*a* b* space known as CIELAB, has generally replaced the Hunter space for industrial applications although this has been somewhat slower in parts of the food industry where methods established on the Hunter system have economic reasons for its continued use. The improvements in CIELAB are due to the nonlinear cube root transformation of the 1931 tristimulus values, which more approximate the visual spacing of the coloured samples in the Munsell system (MacDougall, 2002).

While CIELAB uses Cartesian coordinates to calculate a color in a color space, CIE LCH uses polar coordinates. This color expression can be derived from CIELAB. The L* defines lightness, C* specifies chroma and h° denotes hue angle, an angular measurement (X-rite, 2000).

Traditional instruments, such as colourimeters and spectrophotometers, have been used extensively in the food industry for colour measurement (Balaban and Odabasi, 2006). Under specified illumination environment, these instruments provide a quantitative measurement by simulating the manner in which the average human eye sees the colour of an object (McCaig, 2002).

Colorimeter

Colorimeter quantifies color by measuring three primary color components of light seen by the human eye, specifically red, green, and blue (also referred to as “RGB”). This “tristimulus” color measurement provides data on how much of these three components are present in the light reflected (solids) or transmitted (typically liquids) by a food product. Such data may be used, for example, to adjust the color components in a prepared food or beverage recipe to improve “eye appeal,” gauge “doneness” and, in fresh foods, to determine factors such as degrees of ripeness and spoilage in relation to shipping, storage, shelf-life, palatability and disposal cycles. Although there is no strict demarcation line where the benefits of colorimetry in foods ends, it should be recognized that it measures color much the same as the human eye. That is, secondary and tertiary colors such as orange, yellow, violet, tans, browns, etc. are not individually quantifiable. This leaves a variability factor that can hamper the consistent reproduction of a desired color in prepared food products formulated for a specific, consistently produced look (KM, 2012).

Spectrophotometer

Spectrophotometry, a scientific “step up” so to speak, is presently the most precise and accurate technique for the measurement, formulation, and quality control of desired colors in prepared food products. Spectrophotometer instruments measure the spectral reflectance or transmittance of an object across the full spectrum of human visible light wavelengths, 400 nm to 700 nm (nanometers), enabling precise specification of any desired color. Spectrophotometers offer greater specificity, making them the instruments of choice for food product color formulation, specification of standards and tolerances, inter-plant color communication, and color quality control in processing operations (KM, 2012). Spectrophotometry or “measurement of the spectrum” allows us to determine the relative way in which the total energy in a beam of light is divided among the wavelengths it contains. The spectrophotometer breaks down the radiation reflected or transmitted by the measured object surface into its component wavelengths from short waves (about 400 nm) to long (about 700 nm). The ray reflected from the object surface is then compared with the ray reflected from a virtually perfect diffusing surface in the spectrophotometer. The differences in their relative reflectances across the
spectrum are recorded and visualized as a curve. By computation the spectrophotometry curve can be translated into colorimetric terms, such as CIE, that can be plotted on a chromaticity diagram. The instruments are usually also capable of converting the measurements into the notations of some colour order system, such as the CIE Lab or Hunter Lab (Urland, 1999). There a number of spectrophotometer used for food color measurement (Table 2).

**Table 1: Differences between colorimeter and spectrophotometer**

<table>
<thead>
<tr>
<th>Colorimeter</th>
<th>Spectrophotometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>An instrument for psychophysical analysis—provides measurements that correlate with human eye-brain perception. Colorimetric data directly read and provided as tristimulus values (XYZ, L, a, b, etc.).</td>
<td>An instrument for physical analysis—provides wavelength-by-wavelength spectral analysis of the reflecting and/or transmitting properties of objects without interpretation by a human. Can indirectly calculate psychophysical (colorimetric) information.</td>
</tr>
<tr>
<td>Consists of sensor and simple data processor.</td>
<td>Consists of sensor plus data processor or computer with software.</td>
</tr>
<tr>
<td>Has a set illuminant and observer combination, usually C/2°.</td>
<td>Has many available illuminant/observer combinations that can be used for calculating tristimulus data and metamerism index.</td>
</tr>
<tr>
<td>Isolates a broad band of wavelengths using a tristimulus absorption filter.</td>
<td>Isolates a narrow band of wavelengths using a prism, grating, or interference filter.</td>
</tr>
<tr>
<td>Is generally rugged and a less complex instrument than a spectrophotometer.</td>
<td>Is a more complex instrument than a colorimeter.</td>
</tr>
<tr>
<td>Works well for routine comparisons of similar colors and for adjustment of small color differences under constant conditions. Optimal for quality inspection.</td>
<td>Works well for color formulation, measurement of metamerism, and variable illuminant/observer conditions. Optimal for both quality inspection and research and development.</td>
</tr>
</tbody>
</table>

**Source:** Hunterlab, 2008b

**Conclusion**

Modern food color spectrophotometers have a great number of functions. Spectrophotometers are expensive and therefore the careful choice must be made.

Instrumental color measurement is widely employed in paint, print and textile industry. There are a good number of spectrophotometers available for these industries and a few of them are low cost (under one lac rupees). For food and dairy industry, only few spectrophotometers are available in the market. And the cost of the entry level models is around 8-9 lac rupees. There is a need to develop low cost color measuring instruments as per the requirement of food industry. Another researchable
issue is to modify and adopt low cost spectrophotometers which have been design for paint or print industry.

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KM. 2012. Food Industry color control. Konica Minolta, New Jersy, USA. pg. 1
Urland, A. 1999. Colour specification and measurement. ICCROM, Rome, Italy. pg.13
Table 2: Comparison of various spectrophotometers used for food color measurement

<table>
<thead>
<tr>
<th>Measurement Principle</th>
<th>Hunterlab Color flex</th>
<th>Hunterlab Labscan</th>
<th>X-rite Color i7</th>
<th>Konica Mintola CM5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Measurement Principle</strong></td>
<td>Dual-beam</td>
<td>Dual-beam</td>
<td>Tri-beam</td>
<td>Dual-beam</td>
</tr>
<tr>
<td><strong>Measurement mode</strong></td>
<td>Reflectance</td>
<td>Reflectance</td>
<td>Reflectance, Transmittance, haze</td>
<td>Reflectance, Transmittance</td>
</tr>
<tr>
<td><strong>Color scales</strong></td>
<td>CIE L<em>a</em>b*, Hunter Lab, CIE L<em>C</em>h, CIE Yxy, CIE XYZ</td>
<td>CIE L<em>a</em>b*, CIE LCh, Hunter Lab, Rdab, RxRyRz, XYZ, Yxy</td>
<td>CIE L<em>a</em>b*, CIE L<em>C</em>h*, Hunter Lab, CIE (XYZxy</td>
<td>L<em>a</em>b*, L<em>C</em>h*, Hunter Lab, Yxy, XYZ, Munsell</td>
</tr>
<tr>
<td><strong>Illuminants</strong></td>
<td>A, C, D50, D55, D65, D75, F2, F7, F11</td>
<td>A, C, D50, D55, D65, D75, F2, F7, F11, TL84, Ultralume 3000</td>
<td>D50, D55, D65, D75, F2, F7, F11, C, A, Horizon (GretagMacbeth), TL84, Ultralume 3000</td>
<td>A, C, D50, D65, F2, F6, F7, F8, F10, F11, F12, ID50, ID65</td>
</tr>
<tr>
<td><strong>Observers</strong></td>
<td>2° and 10°</td>
<td>2° and 10°</td>
<td>2° and 10°</td>
<td>2° and 10°</td>
</tr>
<tr>
<td><strong>Geometry</strong></td>
<td>45° illumination and 0° viewing</td>
<td>0° illumination, 45° circumferential</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td><strong>Detector</strong></td>
<td>256-element diode array</td>
<td>256-element diode array</td>
<td>N.A.</td>
<td>Dual 40-element silicon photodiode arrays</td>
</tr>
<tr>
<td><strong>Spectral Range</strong></td>
<td>400 nm - 700 nm</td>
<td>400 nm - 700 nm</td>
<td>360 to 750 nm</td>
<td>360 nm to 740 nm</td>
</tr>
<tr>
<td><strong>Light Source</strong></td>
<td>Pulsed Xenon Lamp</td>
<td>Pulsed Xenon Lamp</td>
<td>Pulsed Xenon Lamp</td>
<td>Pulsed Xenon Lamp</td>
</tr>
</tbody>
</table>
Food Laws and Regulatory Issues of Traditional Dairy Products

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Introduction

Food laws and Regulatory frameworks all around the globe have been primarily set up to achieve the objective of ensuring food safety and protection of consumer interests in relation to food, taking into account in particular the diversity in the supply of food including traditional products, whilst ensuring the effective functioning of the internal market.

‘Food law’ means the laws, regulations and administrative provisions governing food in general, and food safety regulation, in particular, whether at Community or national level; it covers any stage of production, processing and distribution of food, and also of feed produced for, or fed to, food producing animals. Food law shall pursue one or more of the general objectives of a high level of protection of human life and health and the protection of consumers' interests, including fair practices in food trade, taking account of, where appropriate, the protection of animal health and welfare, plant health and the environment.

Traditional milk products represent the most prolific segment of our Indian Dairy Industry. However, despite the widespread popularity and acceptability of traditional milk products in the Indian milieu, the organized sector has still not been able to fully trap their market potential for many reasons. Perhaps that is the reason; many traditional Indian dairy products are still not covered under various National Standards. Only a handful of traditional dairy products are embraced under various National Standards. Table 2 gives a list of traditional dairy products covered under various standards.

General safety requirements under food law:

1. Food shall not be placed on the market if it is unsafe.

2. Food shall be deemed to be unsafe if it is considered to be:

   (a) Injurious to health;

   (b) Unfit for human consumption.

3. In determining whether any food is unsafe, regard shall be had:

   (a) To the normal conditions of use of the food by the consumer and at each stage of production, processing and distribution, and

   (b) to the information provided to the consumer, including information on the label, or other information generally available to the consumer concerning the avoidance of specific adverse health effects from a particular food or category of foods.

4. In determining whether any food is injurious to health, regard shall be had:

   (a) not only to the probable immediate and/or short-term and/or long-term effects of that food on the health of a person consuming it, but also on subsequent generations;

   (b) to the probable cumulative toxic effects;
(c) to the particular health sensitivities of a specific category of consumers where the food is intended for that category of consumers.

5. In determining whether any food is unfit for human consumption, regard shall be had to whether the food is unacceptable for human consumption according to its intended use, for reasons of contamination, whether by extraneous matter or otherwise, or through putrefaction, deterioration or decay.

6. Where any food which is unsafe is part of a batch, lot or consignment of food of the same class or description, it shall be presumed that all the food in that batch, lot or consignment is also unsafe, unless following a detailed assessment there is no evidence that the rest of the batch, lot or consignment is unsafe.

7. Food that complies with specific Community provisions governing food safety shall be deemed to be safe insofar as the aspects covered by the specific Community provisions are concerned.

8. Conformity of a food with specific provisions applicable to that food shall not bar the competent authorities from taking appropriate measures to impose restrictions on it being placed on the market or to require its withdrawal from the market where there are reasons to suspect that, despite such conformity, the food is unsafe.

9. Where there are no specific Community provisions, food shall be deemed to be safe when it conforms to the specific provisions of food law of the State in whose territory the food is marketed.

Overview of food regulatory system

Food regulations and standards differ significantly between countries and across larger jurisdictional organizations (e.g. Codex Alimentarius, USA Food and Drug Administration, in India FSSAI) but generally, before a “new” food can be placed on the market, specific authorization will need to be obtained from the relevant regulator. The process of authorization will depend on whether the food or ingredient is regarded as new, novel, traditional or non-traditional (i.e. does not have a significant history of consumption in that market).

Dairy and food processors should have a basic understanding of regulations to ensure that they are in compliance. Regulations are of two basic types. A substantive regulation, which carries the same weight as a law, defines what may or may not be done. For example, labeling regulations define precisely how a label is to be prepared. The second type, an interpretive regulation, is enacted to address a law but allows some leeway in how to comply. Such regulations use the word “should.” Since it takes a long time to develop regulations and pass laws, it may take years for the law to be enacted.

Regulatory issues and food laws in India

The dairy and food business in India are regulated by the Food Safety and Standards (FSSAI) Regulations 2011, notified by the Gazette of India dated 1st Aug 2011, these Regulations shall come in force on or after 5th Aug 2011:

1. FSS (Licensing and Registration of Food Businesses) regulation, 2011
2. FSS (Packaging and Labelling) regulation, 2011
3. FSS (Food product standards and Food Additives) regulation, 2011 (part I)
4. FSS (Food product standards and Food Additives) regulation, 2011 (part II)
5. FSS (Prohibition and Restriction on Sales) regulation, 2011
The Food Safety and Standards Authority of India (FSSAI) has been established under Food Safety and Standards Act, 2006 which consolidates various Acts & Orders (Prevention of Food Adulteration Act, 1954.; Fruit Products Order, 1955.; Meat Food Products Order, 1973.; Vegetable Oil Products (Control) Order, 1947.; Edible Oils Packaging (Regulation) Order, 1988.; Solvent Extracted Oil, De-Oiled Meal and Edible Flour (Control) Order, 1967.; Milk and Milk Products Order, 1992. Etc.,) that have hitherto handled food related issues in various Ministries and Departments. FSSAI has been created for laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, sale and import to ensure availability of safe and wholesome food for human consumption. The Act also aims to establish a single reference point for all matters relating to food safety and standards, by moving from multi-level, multi-departmental control to a single line of command. To this effect, the Act establishes an independent statutory Authority. There are different chapters under the FSS regulations, each chapters consists of parts and sub-parts and regulations, each regulations deals separately with different aspects. Chapter (Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011) deals with the food products standards and under this part Chapter 2 meant for dairy products and its analogues as shown below.

**Table 1. Milk and milk products covered under FSSAI Regulations**

<table>
<thead>
<tr>
<th>Regulation No.</th>
<th>Product name</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.1</td>
<td>Milk</td>
</tr>
<tr>
<td>2.1.2</td>
<td>Cream</td>
</tr>
<tr>
<td>2.1.3</td>
<td>Malai</td>
</tr>
<tr>
<td>2.1.4</td>
<td>Dahi or Curd</td>
</tr>
<tr>
<td>2.1.5</td>
<td>Chhana or Paneer</td>
</tr>
<tr>
<td>2.1.6</td>
<td>Cheese</td>
</tr>
<tr>
<td></td>
<td>1. Cheese</td>
</tr>
<tr>
<td></td>
<td>2. Processed Cheese</td>
</tr>
<tr>
<td></td>
<td>3. Processed Cheese Spread</td>
</tr>
<tr>
<td></td>
<td>4. Cheddar Cheese</td>
</tr>
<tr>
<td></td>
<td>5. Danbo Cheese</td>
</tr>
<tr>
<td></td>
<td>6. Edam Cheese</td>
</tr>
<tr>
<td></td>
<td>7. Gouda Cheese</td>
</tr>
<tr>
<td></td>
<td>8. Havarti Cheese</td>
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<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>9.</td>
<td>Tilsiter</td>
</tr>
<tr>
<td>10.</td>
<td>Cottage Cheese and Creamed Cottage Cheese</td>
</tr>
<tr>
<td>11.</td>
<td>Cream Cheese (Rahmfrischkase)</td>
</tr>
<tr>
<td>12.</td>
<td>Coulommiers Cheese</td>
</tr>
<tr>
<td>13.</td>
<td>Camembert Cheese</td>
</tr>
<tr>
<td>14.</td>
<td>Brie Cheese</td>
</tr>
<tr>
<td>15.</td>
<td>Saint Paulin</td>
</tr>
<tr>
<td>16.</td>
<td>Samsoe</td>
</tr>
<tr>
<td>17.</td>
<td>Emmentaler</td>
</tr>
<tr>
<td>18.</td>
<td>Provolone</td>
</tr>
<tr>
<td>19.</td>
<td>Extra Hard Grating Cheese</td>
</tr>
<tr>
<td>2.1.7</td>
<td>Dairy Based Desserts/Confections</td>
</tr>
<tr>
<td>1.</td>
<td>Ice Cream, Kulfi, Chocolate Ice Cream or Softy Ice Cream</td>
</tr>
<tr>
<td>2.</td>
<td>Dried Ice Cream Mix/Dried Frozen Dessert/Confection</td>
</tr>
<tr>
<td>3.</td>
<td>Frozen Dessert/Frozen Confection</td>
</tr>
<tr>
<td>4.</td>
<td>Milk Ice or Milk Lolly</td>
</tr>
<tr>
<td>5.</td>
<td>Khoya</td>
</tr>
<tr>
<td>2.1.8</td>
<td>Evaporated/Condensed Milk &amp; Milk Products</td>
</tr>
<tr>
<td>1.</td>
<td>Evaporated milk</td>
</tr>
<tr>
<td>2.</td>
<td>Sweetened Condensed Milk</td>
</tr>
<tr>
<td>3.</td>
<td>Milk Powder</td>
</tr>
<tr>
<td>2.1.9</td>
<td>Foods for Infant Nutrition</td>
</tr>
<tr>
<td>1.</td>
<td>Infant Milk Food</td>
</tr>
<tr>
<td>2.</td>
<td>Infant formula</td>
</tr>
<tr>
<td>3.</td>
<td>Milk-cereal based complementary food milk</td>
</tr>
<tr>
<td>4.</td>
<td>Processed cereal based complementary food commonly called as weaning food or supplementary food</td>
</tr>
<tr>
<td>5.</td>
<td>Follow-Up Formula-Complementary Food</td>
</tr>
<tr>
<td>2.1.10</td>
<td>Butter, Ghee &amp; Milk Fats</td>
</tr>
<tr>
<td>1.</td>
<td>Butter</td>
</tr>
<tr>
<td>2.</td>
<td>Ghee</td>
</tr>
<tr>
<td>3.</td>
<td>Milkfat / Butter oil and Anhydrous Milk fat / Anhydrous Butter oil</td>
</tr>
</tbody>
</table>
A standard can be defined as a documented specification for properties of manufactured goods. It is a specification that has been worked out with scientific facts. The specifications are expressed in terms of some standard measurement units that are understandable and easy to follow. The enforcement of standard of particular food item gives the consumer a satisfaction that the product has defined specifications. Consumers around the world in general have the following concerns about food and most of these concerns can be addressed satisfactorily if there is a mechanism of enforcement of certain defined standards

1. Value for money is questionable
2. Adulteration and improper handling of foods lower nutritional quality
3. Information of the label is inadequate, not comparable and sometimes difficult to read
4. Doubts on the implementation of safety measures (good agricultural and manufacturing practices) being adopted during handling of foods
5. Lack of concern for environment
6. Doubts on the activities of enforcement agencies

Table 2. Traditional dairy products covered under various standards

<table>
<thead>
<tr>
<th>Name of the product</th>
<th>Type of Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FSSAI</td>
</tr>
<tr>
<td>Khoa*</td>
<td>√</td>
</tr>
<tr>
<td>Burfi*</td>
<td>-</td>
</tr>
<tr>
<td>Gulabjamun*</td>
<td>-</td>
</tr>
<tr>
<td>Channa*</td>
<td>√</td>
</tr>
<tr>
<td>---------</td>
<td>---</td>
</tr>
<tr>
<td>Canned Rasogolla*</td>
<td>-</td>
</tr>
<tr>
<td>Paneer</td>
<td>√</td>
</tr>
<tr>
<td>Chakka &amp; Shrikhand*</td>
<td>√</td>
</tr>
<tr>
<td>Dahi*</td>
<td>√</td>
</tr>
<tr>
<td>Kulfi*</td>
<td>√</td>
</tr>
<tr>
<td>Butter</td>
<td>√</td>
</tr>
<tr>
<td>Ghee</td>
<td>√</td>
</tr>
</tbody>
</table>

*Code for hygienic conditions for production, transport, storage and distribution has been defined under BIS and standard in the context is available

Table 3. List of standards on milk & milk products not available under FSSAI regulations, 2011

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soy Milk</td>
</tr>
<tr>
<td>2</td>
<td>Flavoured Soy Milk</td>
</tr>
<tr>
<td>3</td>
<td>Tofu</td>
</tr>
<tr>
<td>4</td>
<td>Fat Filled Powder</td>
</tr>
<tr>
<td>5</td>
<td>Filler Cheese/Sauce/Cheese Dips</td>
</tr>
<tr>
<td>7</td>
<td>Kheer</td>
</tr>
<tr>
<td>8</td>
<td>Beverage Whitener</td>
</tr>
<tr>
<td>9</td>
<td>Dairy Based Beverage Mix</td>
</tr>
<tr>
<td>10</td>
<td>Aseptic Beverage Creamer</td>
</tr>
<tr>
<td>11</td>
<td>Frozen Non Dairy Whipping Cream/Non Dairy Whip Topping</td>
</tr>
<tr>
<td>12</td>
<td>Frozen Vegetable Fat based Cooking Cream</td>
</tr>
</tbody>
</table>

The standards for traditional dairy products are enforced through different agencies as mentioned in Table 2. Sometimes the standards from one agency may not tally with the other resulting in improper implementation. This is the issues in the traditional dairy products. Agriculture Marketing
AGMARK deals with ghee and butter. BIS certification for products is voluntary but under FSSAI, it is compulsory for some products.

**AGMARK Grading**

Literally, AGMARK is an insignia – AG for ‘Agricultural’ and MARK for ‘marking’. Agricultural commodities do not normally conform to any specific standard but vary considerably and a strict control over their quality has to be maintained. In the case of products like ghee and butter there is a vast variation in quality and analytical parameters between the different regions of the country. These variations could arise due to the differences in crops, oil seeds, etc. used for feeding cattle. For example the Reichert Meissl (R.M) value of ghee produced in cotton tract areas like Saurashtra, etc. is much lower than the minimum permissible limits specified for normal ghee. In such cases there is a possibility of even pure ghee being rejected as adulterated. To overcome these problems and to ensure that the consumer gets a pure, unadulterated product, the Directorate of Marketing and Inspection introduced the quality control and standardization of ghee and butter in 1938. The grading of ghee under Agmark was introduced under the provisions of the Agricultural Produce (Grading and Marketing) Act, 1937. This act which is permissive in nature provides the grading of ghee and butter on a voluntary basis.

The Directorate of Marketing and Inspection (DMI) issues a certificate of authority to the packer after confirming that the product conforms to the rigid quality specifications of the Agmark. The testing is done either at the producer’s own established laboratory or at the State Grading Laboratory by a panel of chemists approved by the Directorate of Marketing and Inspection. After the ghee is packed, labeled and marketed, samples may be drawn occasionally from the market and tested at the Regional Analytical Laboratory.

Ghee is graded either as Special’ and ‘General’, which are represented by two differently coloured labels. The only difference in the grades is in the maximum limit of free fatty acids (oleic), which in special grade (Red label) ghee is limited to 1.4% and in general grade (Green label) to 2.5%. The other parameters defined are butyro-refractometer reading at 40°C, moisture content, Reichert-Meissl value, Polenske value, Baudouin test etc. Similarly, two grades of butter viz., pasteurized table butter and unpasteurized table butter have been prescribed on the basis of flavour, aroma, body and texture, colour, moisture content, acidity curd, etc.

**Bureau of Indian Standards**

Bureau of Indian Standards (earlier ISI) had started the work with respect to formulation of standards for various food products since its inception in 1947. Indian Standards are formulated by the technical committee comprising experts from the Industry, Govt. departments, testing laboratories, users and consumers. National Standards are evolved through a consensus opinion of the members comprising of a technical committee. In this context, the Bureau of Indian Standards, which is the national organization for standardization in our country has so far formulated over 16000 Indian Standards.

BIS has published Indian Standards for all the above products to take care of the quality as well as safety and health aspects of the consumers. Such standards also emphasize on the requirement of hygienic conditions to be maintained during storage of raw materials, manufacturing and processing, packaging and storage of the food products. One advantage with BIS standard is that, these standards are complete in every sense as they even mention the analytical methods to be followed for testing of such milk products. BIS has also defined Code for hygienic conditions for production, transport,
storage and distribution of some indigenous milk products such as khoa & khoa based sweets, channa & channa based sweets, dahi, kulfi and shrikhand.

BIS also operates a Certification Marks Scheme under the BIS Act, 1986 to help the consumer identify products the quality of which is assured by the Bureau. Under the scheme, interested manufactures are granted licenses to mark their products with the Bureau’s Standard Mark known as ISI after the Bureau satisfies itself to that affect.

Table 4. List of Standards for Milk and Milk Products under Mandatory BIS Certification

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parent Act</th>
<th>Rules/QC Order</th>
<th>Notification</th>
<th>Implementing Authority</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Food Safety &amp; Standards Act, 2006</td>
<td>Food Safety &amp; Standards (Prohibition &amp; Restriction on Sales) Regulation, 2011</td>
<td>Ministry of Health and Family Welfare, Dept. of Health, Notification dated : 1 Aug 2011 Date of Implementation: 5 Aug 2011</td>
<td>Food (Health) Authority of the State</td>
</tr>
<tr>
<td>1.</td>
<td>IS 1165</td>
<td>Milk Powder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>IS 1166</td>
<td>Condensed milk, partly skimmed and skimmed condensed milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>IS 1656</td>
<td>Milk-cereal based weaning foods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>IS 11536</td>
<td>Processed cereal based complementary foods for infants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>IS 12176 (Part 1)</td>
<td>Sweetened ultra high temperature treated condensed milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>IS 13334 (Part 1)</td>
<td>Skimmed milk powder, standard grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>IS 13334 (Part 2)</td>
<td>Skimmed milk powder, extra grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>IS 14542</td>
<td>Partly skimmed milk powder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>IS 14433</td>
<td>Infant milk substitute, milk protein based</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>IS 15757</td>
<td>Follow-up Formula – Complimentary Foods</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Codex Standards and traditional dairy products

The different sets of standards arising from the spontaneous and independent development of food laws and standards by different countries inevitably gave rise to trade barriers that were of increasing
concern to food traders in the early twentieth century. Trade associations that were formed as a reaction to such barriers pressured governments to harmonize their various food standards so as to facilitate trade in safe foods of a defined quality. The International Dairy Federation (IDF), founded in 1903, was one such association. Its work on standards of milk and milk products later provides a catalyst in the establishment of Codex Alimentarius Commission and in the setting of its procedures for elaborating standards.

The Codex Alimentarius Commission is responsible for making proposals to, and shall be consulted by, the Director-General of the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) on all matters pertaining to the implementation of the joint FAO/WHO Standard Programme.

In addition to commodity standards, the Codex Alimentarius include general standards, which has across-the-board application to all foods and are not product specific. There are general standards or recommendations for:

- Food Labeling
- Food Additives
- Contaminants
- Method of analysis and sampling
- Food hygiene
- Nutrition and foods for special dietary uses
- Food import and export inspection and certifications systems
- Residues of veterinary drugs in foods
- Pesticide residues in foods.

Although it is still a distant dream that traditional dairy products may find suitable place in the listing of Codex Standards as commodity standards, general standards may be followed for the harmonization purpose. For export purposes, general standards listed above can be adopted.

Conclusion

India’s traditional dairy sector is poised for rapid expansion with the result of application of modern process technologies in the production of Indian sweets. The need of the market will determine the change in technology that will be required in the future. Fast changes in socio-economic environment will drive the requirements for traditional dairy products to be processed and packaged in new forms. Although demand for traditional dairy products far exceed that of western type of dairy products, it present only handful of the traditional dairy products are covered under various standards. The role of standards in supporting and facilitating the smooth functioning of the chain of processes and also embedding quality in the system cannot be underestimated. Standard provide the needed guidelines to the entrepreneurs in ensuring the right practices to be followed during various stages, such as storage, transportation, selection of raw material, processing and packing and also pinpoint the specifications which should be adhered to, in order to promote quality and safety of the end product. So for integrated speedy development of dairy sector, standardization has a vital role to play.
In-Vitro Techniques for Evaluation of Antioxidant Potential of Functional Dairy Foods

Rajesh Kumar
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National Dairy Research Institute, Karnal-135001

Introduction

Free radicals released during oxidative stress pose major endogenous damage in the biological system. This type of damage is often associated with various degenerative diseases and disorders such as cancer, cardiovascular disease, reduced immune function and aging. The body has its own defence system against these free radicals based on antioxidative enzymes, as well as low molecular weight mass non enzymatic antioxidant compounds. These defence systems are not effective enough to totally prevent the damage, and therefore, food supplements containing antioxidants may be used to help the human body to reduce oxidative damage.

Over the past 25 years, antioxidant assays have shifted considerably from the initial assay focused on elucidating endogenous activity to massive efforts to screen large numbers of biological materials for free radical scavenging activity that presumably will signal potential for in vivo preventative or therapeutic effects, namely, to identify super foods and super-nutraceuticals that should be consumed for improved health. Currently, the potential health benefits of the phytochemicals and their ability to be incorporated into dairy foods as nutraceuticals has received considerable interest in food industry.

Methods to analyze antioxidant activity

Due to the complexity of oxidative processes occurring in food or biological systems as well as the different antioxidative mechanisms by which various compounds may act, finding one method that can characterize the overall antioxidative potential of food is not an easy task. A broad variety of in-vitro techniques has been developed for the detection of antioxidants on the basis of different antioxidative mechanism under variable conditions reflecting the multifunctional properties of antioxidants in both physiological and food-related antioxidation processes. Antioxidant assays may be broadly classified as the electron transfer (ET) - and hydrogen atom transfer (HAT) - based assays.

HAT-based assays

HAT-based assays measure the capability of an antioxidant to quench free radicals (generally, peroxyl radicals considered to be biologically more relevant) by H-atom donation. HAT-based assays include oxygen radical absorbance capacity (ORAC) assay, TRAP assay using R- phycoerythrin as the fluorescent probe, crocin bleaching assay using 2,2'-azobis(2- midinopropane) hydrochloride (AAPH) as the radical generator, and β-carotene bleaching assay

ET-based assays

In most ET-based assays, the antioxidant action is simulated with a suitable redox-potential probe, namely, the antioxidants react with a fluorescent or coloured probe (oxidising agent) instead of peroxyl radicals. Spectrophotometric ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes colour when reduced. The degree of colour change (either an increase or decrease of absorbance of the probe at a given wavelength) is correlated to the concentration of antioxidants in the sample. 2,2’-Azinobis-(3- ethylbenzothiazoline-6-sulfonic acid) (ABTS) / Trolox-equivalent antioxidant capacity (TEAC) and 2,2-di(4-tert-octylphenyl)-1-
picrylhydrazyl (DPPH) are decolourisation assays, whereas in Folin total phenols assay, ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC), there is an increase in absorbance at a pre specified wavelength as the antioxidant reacts with the chromogenic reagent [i.e., in the latter two methods, the lower valencies of iron and copper, namely, Fe(II) and Cu(I), form charge transfer complexes with the corresponding ligands, respectively.

ET-based assays generally set a fixed time for the concerned redox reaction and measure thermo-dynamic conversion (oxidation) during that period. ET-based assays include ABTS/TEAC, DPPH, Folin–Ciocalteu reagent (FCR), FRAP, ferricyanide, and CUPRAC using different chromogenic redox reagents with different standard potentials. Although the reducing capacity of a sample is not directly related to its radical scavenging capability, it is a very important parameter of antioxidants.

Trolox equivalent antioxidant capacity (TEAC) assay: The TEAC assay based on the reduction of coloured cation radical of 2, 2’-azinobis(3-ethylbenzothiazoline-6-sulphonate) (ABTS+) ABTS), which has a characteristic long-wavelength absorption spectrum showing maxima at 660, 734 and 820 nm and the antioxidant capacity is quantified as the concentration of water soluble vitamin E analogue, Trolox, that produces the same effect as the sample. ABTS + test is the most popular among the indirect assays. ABTS + is stable, and it reacts energetically with an H-atom donor such as phenolics, being converted into a non-coloured form of ABTS +. The quantity of ABTS + consumed due to reaction with phenolic – containing sample, expressed as trolox equivalent antioxidant capacity (TEAC). It is applicable to both hydrophilic and lipophilic compounds. The advantage of ABTS + method is its relative simplicity. The result of determination of TEAC is expected to be dependent on the time of incubation as well as on the ratio of sample quantity to ABTS + concentration. ABTS + method is successfully employed for antioxidant capacities of bovine milk, whey, skim, milk.

DPPH radical scavenging activity (RSA) method: This method is based on scavenging of the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) from the antioxidants, which produces a decrease in absorbance at 515 nm. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of colour. The decrease in colour has been correlated to a dose response curve with a standard antioxidant as in the TEAC assay. These methods may be useful for screening antioxidants, but antioxidant effectiveness in foods should be studied by other methods, since their activity in foods is dependent on a variety of factors including polarity, solubility, and metal-chelating activity.

Ferric reducing ability of plasma (FRAP) assay: It is a technique to determine the total antioxidant power interpreted as the reducing capability. The FRAP assay measures antioxidant capacity by the reduction of the ferric tripyridyltriazine complex to the blue ferrous complex with an increase in absorbance at 593 nm which is proportional to the combined (total) ferric reducing/antioxidant power (FRAP value) of the antioxidants in the sample.

Oxygen radical absorbance capacity (ORAC) assay: The oxygen radical absorbance capacity (ORAC) assay based on quenching of fluorescence from the protein β-phycoerythrin by radicals. Peroxyl radicals, which are intermediates in lipid oxidation, may be generated by azo compounds such as lipophilic α, α'-azobisisobutyronitrile and 2, 2’-azobis(2,4-dimethylvaleronitrile) and the hydrophilic 2, 2’ azobis (2-amidinopropane) dihydrochloride. These initiators have been found to be useful in the study of the kinetics of radical scavenging in various systems because radicals are generated at a reproducible and constant rate depending on temperature. These azo compounds are also artificial and not natural to food or other biological systems.
Lipid oxidation: Evaluation of the ability to inhibit or halt lipid oxidation in model systems is based on measuring changes in the concentration of compounds being oxidized, on depletion of oxygen or on formation of oxidation products. Quantification of the loss of reactants (oxygen, unsaturated fatty acids), formation of free radicals, and formation of oxidation products may be the most appropriate marker depending on the stage of oxidation. Standard methods for measurement of lipid oxidation as peroxide value (POV, formation of lipid hydroperoxides), thiobarbituric acid reactive substances (TBARS, formation of secondary oxidation products) among others are used. The TBARS test assay is based on the measurement of malondialdehyde (MDA) formed as a consequence of lipid peroxidation and the test can be conducted with subcellular membrane preparations or intact cells. The test is unspecific and does not only measure the formation of malondialdehyde, but also other oxo-compounds.

Cell culture based assay: A general major problem associated with the use of the chemical analytical methods for antioxidant activity is that they are conducted under non physiological conditions. Accordingly, the results cannot be extrapolated to the in vivo situation. A step closer to human situation is the use of intact cells which can be challenged with reactive oxygen species (ROS) generating chemicals or radiation in the absence and presence of putative antioxidants. The extracellular release of superoxide or intracellular ROS and superoxide production, for example, can be measured by a plate reader assay with cells in situ or by flow cytometric analysis. Cell culture models can also be used to evaluate cytotoxicity of antioxidative compounds at concentrations to be used to exert the desired bioactivity in the body, as well as to study the potential to inhibit intracellular oxidation and to reduce inflammatory responses. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which measures the metabolic activity of cells through oxidation–reduction activities of mitochondria, is often used to measure the viability of cells during cytotoxicity assays.

Current status

Both conceptual and technical limitations lie in most current antioxidant assays developed for ease of use and rapid screening of large numbers of materials. All assays in current use were designed on the assumption that antioxidant action in vivo proceeds by the same free radical scavenging shown in solution, yet in vivo radical scavenging associated with absorbed antioxidants (as opposed to in vitro cell culture) has not been demonstrated. Very low bioavailability, absorption, distribution, and unknown metabolism of antioxidants impose severe limitations on what reactions these compounds can mediate competitively in vivo; indeed, low absorption may render the chemistry measured in the assays irrelevant outside of the gastrointestinal tract. Polyphenols are chemically reactive and undergo reactions other than radical scavenging, one of which is binding to proteins; these are active in vivo and must be considered when designing test diets and applications. Most current in vitro assays measure inaccurate chemistry in concentration ranges (both absolute and relative) many orders of magnitude higher than ever seen in vivo. Some radical assays use molecular targets (e.g., sterically hindered >N•) that do not represent chemistry of in vivo targets. Reaction times in assays run from 4 minutes to many hours, while the lifetimes of oxygen radicals normally are being combated in vivo and in foods are very short.

Conclusion

The increasing interest gained by antioxidants is due to the health benefits in preventing the occurrence of oxidative-stress related diseases. Utmost care should be exercised in both the practice and evaluation of methods, ensuring the repeatability/reproducibility of results. All methods are in vitro assays giving summed or integrated parameters rather than individual antioxidant quantifications. The
results obtained from the listed in vitro assays cannot be extrapolated directly to the situation in the human body; however, experimental conditions can be tailored to fit in vivo simulations.

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Laboratory Accreditation- Concept and Its Implementation

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Accreditation is a procedure by which the accrediting body gives formal recognition that a body or organization is competent to carry out specific tasks. The concept of laboratory accreditation was developed to provide a means for third-party certification of the competence of laboratories to perform specific type(s) of testing and calibration. In the past, experience in many interlaboratory studies at national and international level has demonstrated that beside standardized and validated methods, analytical quality assurance plays a key role for the reliability of laboratory results. Introduction of systematic quality assurance procedures (such as laboratory accreditation and GLP in some cases) of the analytical work itself is now a requirement for confidence in laboratories and for the acceptance of results. The globalization of Indian economy and the liberalization policies initiated by the Government in reducing trade barriers and providing greater thrust to exports makes it imperative for laboratories to be at international level of competence.

Laboratory accreditation provides formal recognition to competent laboratories, thus providing a ready means for customers to identify and select reliable testing, measurement and calibration services. To maintain this recognition, laboratories are re-evaluated periodically by the accreditation body to ensure their continued compliance with requirements, and to check that their standard of operation is being maintained. The laboratory may also be required to participate in relevant proficiency testing programs between reassessments, as a further demonstration of technical competence.

Accredited laboratories usually issue test or calibration reports bearing the accreditation body’s logo or endorsement, as an indication of their accreditation. Clients are encouraged to check with the laboratory as to what specific tests or measurements they are accredited for, and for what ranges or uncertainties. This information is usually specified in the laboratory’s scope of accreditation, issued by the accreditation body. The description in the scope of accreditation also has advantages for the customers of laboratories in enabling them to find the appropriate laboratory or testing service.

Laboratory accreditation uses criteria and procedures specifically developed to determine technical competence. Specialist technical assessors conduct a thorough evaluation of all factors in a laboratory that affect the production of test or calibration data. The criteria are based on an international standard called ISO/IEC 17025, which is used for evaluating laboratories throughout the world. Laboratory accreditation bodies use this standard specifically to assess factors relevant to a laboratory’s ability to produce precise, accurate test and calibration data, including the:

- technical competency of staff
- validity and appropriateness of test methods
- traceability of measurements and calibrations to national standards
- suitability, calibration and maintenance of test equipment
testing environment
- sampling, handling and transportation of test items
- quality assurance of test and calibration data

Manufacturing organizations may also use laboratory accreditation to ensure the testing of their products by their own in-house laboratories is being done correctly.

A marketing advantage

Laboratory accreditation is highly regarded both nationally and internationally as a reliable indicator of technical competence. Many industries, such as the construction materials industry, routinely specify laboratory accreditation for suppliers of testing services. Unlike certification to ISO 9001, laboratory accreditation uses criteria and procedures specifically developed to determine technical competence, thus assuring customers that the test, calibration or measurement data supplied by the laboratory or inspection service are accurate and reliable. Many accreditation bodies also publish a directory of their accredited laboratories, which includes the laboratories’ contact details plus information on their testing capabilities. This is another means of promoting a laboratory’s accredited services to potential clients.

A benchmark for performance

Laboratory accreditation benefits laboratories by allowing them to determine whether they are performing their work correctly and to appropriate standards, and provides them with a benchmark for maintaining that competence. Many such laboratories operate in isolation to their peers, and rarely, if ever, receive any independent technical evaluation as a measure of their performance. A regular assessment by an accreditation body checks all aspects of a facility’s operations related to consistently producing accurate and dependable data. Areas for improvement are identified and discussed, and a detailed report provided at the end of each visit. Where necessary, follow-up action is monitored by the accreditation body so the facility is confident that it has taken the appropriate corrective action.

International recognition for your laboratory

Many countries around the world have one or more organizations responsible for the accreditation of their nation’s laboratories. Most of these accreditation bodies have now adopted ISO/IEC 17025 as the basis for accrediting their country’s testing and calibration laboratories. This has helped countries employ a uniform approach to determining laboratory competence. It has also encouraged laboratories to adopt internationally accepted testing and measurement practices, where possible. This uniform approach allows countries to establish agreements among themselves, based on mutual evaluation and acceptance of each other’s accreditation systems. Such international agreements, called mutual recognition arrangements (MRAs), are crucial in enabling test and calibration data to be accepted between these countries. In effect, each partner in such an MRA recognizes the other partner’s accredited laboratories as if they themselves had undertaken the accreditation of the other partner’s laboratories.

ILAC (International Laboratory Accreditation Co-operation) is the peak international authority on laboratory accreditation, with a membership consisting of accreditation bodies and affiliated organizations throughout the world. It is involved with the development of laboratory accreditation practices and procedures, the promotion of laboratory accreditation as a trade facilitation tool, the assistance of developing accreditation systems, and the recognition of competent test and calibration facilities around the globe. In 1996, 44 national bodies signed a Memorandum of Understanding in Amsterdam that provided the basis for the development of the co-operation and the eventual
establishment of a recognition agreement between ILAC member bodies. As part of its global approach, ILAC also provides advice and assistance to countries that are in the process of developing their own laboratory accreditation systems. In conjunction with ILAC, specific regions have also established their own accreditation co-operations, notably in Europe (EAL) and the Asia-Pacific (APLAC). These regional co-operations work in harmony with ILAC and are represented on ILAC’s board of management. India is also a signatory to ILAC Arrangements as well as APLAC MRAs.

The developing system of international MRAs among accreditation bodies has enabled accredited laboratories to achieve a form of international recognition, and allowed data accompanying exported goods to be more readily accepted on overseas markets and thus a step towards elimination of technical barrier to trade. This effectively reduces costs for both the manufacturer and the importer, as it reduces or eliminates the need for products to be retested in another country.

**How does using an accredited laboratory benefit government and regulators?**

Government bodies and regulators are constantly called upon to make decisions related to:

- Protecting the health and welfare of consumers and the public
- Protecting the environment
- Developing new regulations and requirements
- Measuring compliance with regulatory and legal requirements
- Allocating resources, both technical and financial

Government bodies and regulators must have confidence in the data generated by laboratories in order to make these decisions. Using an accredited laboratory can help establish and assure this confidence. If a laboratory is accredited, it means that the laboratory has achieved a prescribed level of technical competence to perform specific types of testing, measurement and calibration activities. The result is assurance that the laboratory is capable of producing data that are accurate, traceable and reproducible - critical components in governmental decision-making.

Using an accredited laboratory benefits government and regulators by:

- Increasing confidence in data that are used to establish baselines for key analyses and decisions
- Reducing uncertainties associated with decisions that affect the protection of human health and the environment
- Increasing public confidence, because accreditation is a recognizable mark of approval
- Eliminating redundant reviews and improving the efficiency of the assessment process (which may reduce costs)
- Purchases received from suppliers are safe and reliable
- Costs associated with laboratory problems, including re-testing, resampling, and lost time are minimized
- False positives and negatives, which can directly affect compliance with regulations, are minimized

Using accredited laboratories also facilitates trade and economic growth because data generated by an accredited laboratory may lead to the more ready acceptance of exported goods in overseas markets. This reduces costs and eases exports and imports, as it reduces or eliminates the need for retesting in another country.
Why is a laboratory’s technical competence as critical to you as a manufacturer, supplier, exporter or customer?

Minimized Risk: Throughout the world today, customers seek reassurance that the products, materials or services they produce or purchase meet their expectations or conform to specific requirements. This often means that the product is sent to a laboratory to determine its characteristics against a standard or a specification. For the manufacturer or supplier, choosing a technically competent laboratory minimizes the risk of producing or supplying a faulty product.

Avoid expensive retesting: Testing of products and materials can be expensive and time consuming, even when they are done correctly the first time. If not done correctly, then the cost and time involved in re-testing can be even higher if the product has failed to meet specifications or expectations. Not only costs go up, but your reputation as a supplier or manufacturer can go down. You can also be held liable for any failure of your product, particularly if it involves public safety or financial loss to a client. Choosing a technically competent laboratory minimizes the chance of retesting being required.

Enhance your customers’ confidence: Confidence in your product is enhanced if clients know it has been thoroughly evaluated by an independent, competent testing facility. This is particularly so if you can demonstrate to them that the laboratory itself has been evaluated by a third party. Increasingly customers are relying on independent evidence, rather than simply accepting a supplier’s word that the product is “fit for purpose”.

Reduce costs and improve acceptance of your goods overseas: Through a system of international agreements technically competent, accredited laboratories receive a form of international recognition, which allows their data to be more readily accepted on overseas markets. This recognition helps to reduce costs for manufacturers and exporters that have their products or materials tested in accredited laboratories, by reducing or eliminating the need for retesting in the importing country.

What types of laboratories can seek accreditation?

Most national accreditation bodies can provide comprehensive accreditation for: facilities undertaking any sort of testing, product or material evaluation, calibration or measurement; private or government laboratories; one-person operations or large multi-disciplinary organizations; remote field operations and temporary laboratories.

Accreditation of food laboratories

A food laboratory may be accredited for the following classes of tests:

- Food Products - Chemical Testing
- Food Products - Microbiological Testing
- Food Products - Micronutrients
- Food Products - Residues
- Food Products - Sensory Evaluation
- Microbiological Condition of Food Processing Factories
- Packaging tests
- Shelf Life testing

Laboratories seeking accreditation for chemical, microbiological and sensory food analyses must be able to demonstrate that they can competently use the methods included in the scope of the
accreditation. If a method is to be used for the official control of foods there are extensive requirements on internal verification, i.e. that the laboratory is able to demonstrate that it can use the method in a way, which enables the analytical task to be solved. The following requirements are examples of factors which laboratories seeking accreditation should pay attention to, since they often are included in a competence assessment:

- the laboratory must have information on the method: is it based on a standard or reference method, or has it been internally developed?
- any deviation in a method as compared to a reference method is fully described and the effects of the deviation have been investigated;
- the method has been verified, e.g. by analysing spiked samples of relevant matrices;
- the laboratory's own written method text is available;
- the method has been in use in the laboratory for a time period of a minimum of three months during which a number of ‘real’ samples of relevant types have been analyzed;
- quality control procedures are in place, e.g. analysis of reference or control materials, or control strains;
- if possible, the laboratory participates in proficiency testing schemes and evaluates, on a continuous basis, the results;
- where relevant, the measurement uncertainty has been estimated and
- if a sensory laboratory, it monitors the performance of individual sensory assessors and of panels.

Documentation showing that the laboratory complies with the requirements presented above must normally be available to the accreditation body and their technical assessors three to four weeks before the assessment. This information is a useful tool for the assessors when they select which parts of an analytical chain are to be assessed. The evaluation of a laboratory's results on the basis of the elements listed above is carried out in order to assess the analytical activities and capabilities of a laboratory to obtain an overall impression of the laboratory. The result should demonstrate whether the laboratory is competent and proficient in the use of the methods for which accreditation is sought.

The standardization and accreditation of sensory quality evaluation methods is a pressing need for the certification of food products, particularly for foods and beverages with specific sensory characteristics, such as those with a protected designation of origin (Lea et al., 1995). A training and qualification process for expert panelists is required. In cheese, panelists score quality of overall sensory parameters (shape, rind, paste colour, eyes, odour, texture, flavour and aftertaste) on a scale, based on how close the product lies to a specific quality standard. Panelists justify the quality scores given on the basis of the absence/presence of specific characteristics in the product and/or the presence of defects. Training requires the prior establishment of references for both characteristics and defects. Qualification trials determine whether or not the expert panelists (both individually and as a panel) are appropriately qualified to carry out the sensory evaluation. This work also shows the quality control maintenance of qualifications for the expert panellist. This approach could be generalized to any type of food and beverage as a reference for the accreditation of sensory quality evaluation methods according to ISO 17025. In this way, each product manufacturer would be able to define its quality standard and, on the basis of this standard, carry out the sensory evaluation using a panel specifically trained for this purpose (Elortondo et al., 2007).
Laboratory accreditation in India

Government of India has authorized National Accreditation Board for Testing and Calibration Laboratories (NABL) as the sole accreditation body for Testing and Calibration laboratories. NABL is an autonomous body under the aegis of Department of Science & Technology, Government of India, and is registered under the Societies Act. NABL has been established with the objective to provide Government, Industry Associations and Industry in general with a scheme for third-party assessment of the quality and technical competence of testing and calibration laboratories. NABL is a full member of both ILAC and APLAC. NABL had undergone the first peer evaluation by a 4 member team of APLAC in July 2000, based on which NABL qualified as an APLAC MRA Partner as well as a Signatory to ILAC Arrangements. NABL was reassessed in July 2004 & July 2008 and as stated earlier the signatory status of NABL within APLAC MRA has been confirmed for further four years i.e. October 2012. NABL provides laboratory accreditation services to laboratories that are performing tests / calibrations in accordance with NABL criteria, which is based on internationally accepted standards and guidelines, such as ISO / IEC Guide 25, ISO / IEC 17025 and EN 45001. These services are offered in a non-discriminatory manner and are accessible to all testing and calibration laboratories in India and abroad, regardless of their ownership, legal status, size and degree of independence. NABL has established its Accreditation System in accordance with ISO/IEC 17011:2004, which is followed internationally. A list of NABL accredited laboratories involved in food testing is available at http://www.nabl-india.org.

Conclusion

It is apparent that laboratory accreditation has an important role to play in establishing the credibility of laboratories. Customers of the providers of analytical data need to be assured about the quality of the data that is being given to them. Experience in many laboratory studies at national and international level in the past has demonstrated that besides standardized and validated methods (although these are key factors); analytical quality assurance plays a key role for the reliability of laboratory results. Introduction of systematic quality assurance procedures of the analytical work itself is now expected to become a requirement for confidence in laboratories and for the acceptance of the results. In this regard laboratory accreditations play an important role in establishing the credibility of analytical laboratories.

Reference


http://www.ilac.org
http://www.nabl-india.org


Introduction

The importance of milk and milk products, in India, has been recognized since Vedic times. Milk is considered to be a complete food as it contains almost all essential nutrients required for human health and growth. Lipids, the most important constituent of milk, play significant role in the nutrition, flavour and physico-chemical properties of milk and milk products. They are also rich source of fat-soluble vitamins (A, D, E & K) and essential fatty acids, apart from having pleasant sensory attributes. Milk fat is easily digestible than other oils and fats. It contains number of components which show anticarcinogenic activity, e.g. sphingomyeline, conjugated linoleic acid, β-carotene etc. So one (especially vegetarians) cannot avoid it in one’s diet. But recent trend, in the society, is against fat-rich dairy products due to the presence of saturated fat & cholesterol as these are known to increase the incidence of coronary heart disease (CHD).

CHD is one of the common causes of heart attack. Through a period of time, many researchers have shown that dietary cholesterol, serum cholesterol and occurrence of coronary heart disease (CHD) have positive correlation. Milk fat contains about 0.25 to 0.40% cholesterol. Consumption of ghee and other fat-rich dairy products makes appreciable contribution to cholesterol intake. Furthermore, some cholesterol oxidation products (COPs) have been reported to be more harmful than cholesterol itself as they are cytotoxic, atherogenic, mutagenic and carcinogenic. Recent wave against cholesterol-containing foods has damaged the image and market growth of fat-rich dairy products. The educated and urban society, in particular, is more conscious about the presence of cholesterol in their diet. This segment of the society is the major consumer of dairy and other food items manufactured by the organized sector. In recent years, demand of cholesterol-free foods has increased tremendously. This has led to increase in market of margarine, vegetable fat filled dairy products, milk fat replaced dairy products, etc.

Owing to the adverse affects of cholesterol on human health, various physical, chemical and biological methods have been developed for reducing cholesterol in foods. These include blending of milk fat with vegetable oils, extraction with organic solvent, adsorption with activated charcoal and saponin, vacuum distillation, molecular distillation, degradation of cholesterol by enzyme (cholesterol oxidase) and removal of cholesterol by supercritical carbon dioxide. Recently, β- cyclodextrin (a starch hydrolysed product) has been effectively used for cholesterol removal from milk, cream, cheese, lard and egg-yolk. Beta cyclodextrin is reported to be non-toxic, non-hygroscopic, chemically stable and edible.

Cholesterol

Cholesterol is a waxy material found in all cells of the body and is a necessary part of cell membranes, some hormones and other body components. In particular, it participates in the formation of myelin sheaths in the brain and peripheral nerves, and modulates the absorption of dietary fats in the intestine. It also acts as a precursor in the biosynthesis of bile acids, steroid hormones and vitamin D, as well as participating in the formation of cell membranes and other biological membranes.
The body makes all the cholesterol it needs; it is not necessary to get any cholesterol from the diet. A high level of cholesterol in the blood is a major risk factor for CHD and heart attack.

**Structure and properties of cholesterol**

The term cholesterol was derived from the Greek words *chole* and *stear*, which mean "bile" and "hard fat," respectively. The origin of the term is a reflection of the fact that the substance was first identified as a hard & white solid in gallstones. Though discovered by Poulletier de la Salle in 1769, cholesterol was not named until 1818, when Michel Chevreul rediscovered it and dubbed it as *cholesterine*, believing that the material was like a fat (Sabine, 1977). Cholesterol is a hydrophobic sterol consisting of a four-ring structure (Figure A) with molecular weight 386.66 and molecular formula: $C_{27}H_{46}O$.

Cholesterol is insoluble in water, sparingly soluble in cold alcohol or petroleum ether, and soluble in hot alcohol and most other organic solvents. Cholesterol melts at 148.5°C. It can be sublimed and distilled under high vacuum. The polar hydroxyl group, which gives cholesterol a slightly hydrophilic nature, can be esterified to a fatty acid, producing cholesterol ester. Both cholesterol and cholesterol ester are important structural components of cell membranes. Cholesterol is also a major determinant of membrane fluidity due to its hydrophobic and hydrophilic regions (Webb *et al.*, 1987).

**Sources of cholesterol in body**

In the body, cholesterol appears through endogenous synthesis and from the diet. Cholesterol synthesis in the body is most active in the liver and intestine and averages 11 mg per kg body weight per day. This equals 770 mg for a 70 kg man on a low (less than 300 mg per day) cholesterol diet (McNamara, 1987). Normally, liver makes 80% of the total blood cholesterol and only 20% comes from the diet (Renner and Gurr, 1991; Allred, 1993). Cholesterol is not considered as an essential dietary nutrient because of its endogenous synthesis. On the other hand, Thomas and Holub (1994) reported that if less dietary cholesterol is consumed, the body compensates by making more cholesterol.

**Digestion, absorption and transportation of cholesterol in the blood**

Digestion and absorption of cholesterol occurs in the small intestine (Grundy, 1983). Cholesterol ester is broken down by a pancreatic cholesterol esterase into free cholesterol, which, absorbed into the cells lining of the intestine. The absorption of endogenous cholesterol (as bile acids) is more efficient than dietary cholesterol absorption.

Fat, including cholesterol, absorbed from the diet, is insoluble in the aqueous medium of the blood. To enable transport through blood system, the various fat components are incorporated into particles called lipoproteins (Grundy, 1983; Mahley and Innerarity, 1983). Lipoproteins consist of a lipid core of triglyceride and cholesterol ester with a surface of mainly phospholipids, protein and some free cholesterol. The four major lipoprotein fractions found in the blood are chylomicrons, very-low density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL).
Chylomicrons are very rich in triglycerides (about 85%) but also contain absorbed cholesterol in the free or esterified form. VLDL is also rich in triglyceride (about 50%) and contains a substantial portion of cholesterol mainly as cholesterol ester. VLDL transport about 15% of the total cholesterol found in the blood.

LDL is enriched in cholesterol and accounts for about 60% of the total blood cholesterol level. It is deposited in artery walls, increasing the buildup of plaque and hence also known as bad cholesterol. HDL carries as much as 20% of the total blood cholesterol level. HDL is thought to be antiatherogenic since it picks up cholesterol from peripheral tissues for delivery to the liver and excretion. Consequently, HDL is called good cholesterol. A better indicator of risk for CHD is the LDL/HDL cholesterol ratio (Thomas and Holub, 1994; Gurr, 1995).

**Synergistic effect of cholesterol with saturated fatty acids on plasma cholesterol level**

Some saturated fatty acids are reported to affect total plasma cholesterol concentration. While, stearic acid has little effect on plasma cholesterol concentration, myristic and palmitic acids have been reported to have the greatest cholesterol raising potential (Hegsted et al., 1965). Some evidence suggests that the effect of myristic and palmitic acids depends on the concomitant intake of dietary cholesterol (National Academy of Sciences, 1989). Such an interaction is clear in several experimental mammals (Spady et al., 1993) and has also been found in some human studies (Fielding et al., 1995). The above reports suggesting an interaction between cholesterol and saturated fat intake; provide a further reason to limit dietary cholesterol.

**Coronary heart disease and atherosclerosis**

Coronary heart disease (CHD) is a condition in which the main coronary arteries which supply blood to the heart are no longer able to supply sufficient blood and oxygen to the heart muscle. CHD, the common cause of heart attack, is one of the most frequent causes of death in the developed and developing countries (AHA, 1989). The rates of mortality due to CHD throughout the world vary. For example, in one study among men aged 40-59 years, the annual incidence rate varied from 15 per 100,000 in Japan to 198 per 100,000 in Finland (Lovegrove and Jackson, 2003). According to Chopra (1997), 2.5 million Indians become victims of heart disease every year, and Indian women are the fastest rising group of coronary patients in the world. He further observed that 33 per 1000 Indians have a greater chance of requiring treatment and intervention for heart disease than either European or Americans.

Atherosclerosis is a silent, painless process and the main cause of CHD characterized by build up of cholesterol-rich fatty deposits on the inner lining of the coronary arteries, which decrease blood flow to the heart muscle by narrowing the arteries substantially (Tabas, 2002). The atherosclerosis plaques usually develop at a point of minor injury in the arterial wall.

**Cholesterol in milk and milk products**

Animal food products like milk and milk products, meat and meat products and eggs are the major sources of cholesterol in our diet. Among these, chicken egg contains highest amount (about 215 mg/egg) of cholesterol. Normally, most of the dieticians believe milk fat as a main source of dietary cholesterol and the main culprit for CHD disease. Cholesterol accounts for 0.25-0.45% of the total lipids in milk. Cholesterol concentrates in the milk fat globule membrane (MFGM). In milk, 80% of the cholesterol is associated with the milk fat globules and the remaining 20% is partitioned into the skim milk phase where it is associated with fragments of cell membrane (Patton & Jensen, 1975). However, any event disrupting the membrane structure, e.g. churning of cream will result in the partial passing of cholesterol alongwith ruptured membrane material to the aqueous phase. Arul et.
al., (1987) studied the distribution of cholesterol in various milk fat fractions viz., solid fraction (m. pt. 39°C), semisolid fraction (m. pt. 21°C) and liquid fraction (m. pt. 12°C) and reported that 80% of the total cholesterol content was present in the liquid fraction of the milk fat. 80-90% of the cholesterol is present in milk in the free form, while 10-20% is esterified (Bindal and Jain, 1973; Wood and Bitman, 1986; Jensen, 1987; Schlimme & Kiel, 1989).

Pantulu and Murthy (1982) observed 8-10 times higher content of cholesterol in whey than in whole milk. Srinivasan (1984) reported the average cholesterol content of cow and buffalo milk as 2.8 and 1.9 mg/g fat, respectively. However, Prasad and Pandita (1990) showed that buffalo milk (20 mg %) contained more cholesterol than cow milk (15.5 mg %). Similarly, they found that dahi from buffalo milk contained more cholesterol as compared to dahi from milk of different breed of cows. In general, dahi had lower cholesterol values than the fresh milk (Ismail and Ahmad, 1978; Prasad and Pandita, 1990). Cholesterol in channa samples exhibited a highly significant variation, being minimum in buffalo, while such species variations were not observed in case of khoa calculated on dry weight basis (Prasad and Pandita, 1990).

Cheese was found to contain 52.3-76.6 (av. 69.3) mg of cholesterol/100 g of cheese and 198-298 (av. 273) mg/100g fat in cheese (Fuke and Matsuoka, 1974). Tylkin et al., (1975) reported 9 times higher cholesterol/g fat in butter milk than butter. Aristova and Bekhova (1976) observed cholesterol content in unsalted butter as 244 mg/100 g. Vyshemirskii et al., (1977) reported that 80-90 % cholesterol initially present in cream passed into butter and 10-20% to butter milk. Masson and Martinez (1984) reported cholesterol content as 177–208 mg/100 g fat in butter. Bindal and Jain (1972) estimated free and esterified cholesterol in Desi ghee, using TLC method and reported their contents as 0.288 and 0.038% and 0.214 and 0.056 % in cow and buffalo ghee, respectively. Prasad and Pandita (1987) observed cholesterol content of ghee prepared from milk of Haryana, Sahiwal and Sahiwal X Friesian cows and from Murrah buffaloes, to be 303, 310, 328 and 240 mg/100 g fat, respectively.

**Factors affecting level of cholesterol in milk and milk products**

**Effect of Species/Breeds**

Bindal and Jain (1973) reported that cow ghee (0.31%) contained higher cholesterol than buffalo ghee (0.267%). Bernolak (1979) observed that cow milk, with 2.8% fat, contained 237 mg total sterols/100 g fat (92.8% cholesterol of total sterols). Prasad and Pandita (1987, 1990) also reported higher cholesterol content in cow ghee compared to that in buffalo ghee. Singh and Gupta (1982) observed that goat ghee contain higher cholesterol (0.236 g/100 g fat) than cow (0.230 g/100 g fat) and buffalo (0.196 g/100 g fat) ghee.

**Effect of Season/Stage of lactation**

Season has also been reported to affect the cholesterol content of milk fat. Treiger (1979) reported that total cholesterol content of cow milk fat ranged from 0.24-0.29 g/100 g fat in spring and 0.18-0.25 g/100 g fat in summer season. Prasad and Pandita (1987, 1990) observed that cholesterol content of ghee was higher in winter than in summer (301 vs 291 mg/100g fat). Krzyzewski et al. (2003) also observed a significantly lower (by about 16%) concentration of cholesterol in milk during winter season. Ghee prepared from milk of old animals (Lal, 1982) and late lactation milk (Nigam, 1989) was found to contain highest level of cholesterol.

**Effect of Heat**

Bector and Narayanan (1975) observed that when cow and buffalo ghee were heated at 225°C for 2 h, respectively 26.1 and 27.3 % of cholesterol was lost. Similarly, Rai and Narayanan (1984) also
reported 28.2 and 49% loss of cholesterol after 12 h of intermittent frying in aluminium and iron container.

Methods of cholesterol removal from milk fat

Since dairy products contain significant amounts of cholesterol, a number of processes for removal of cholesterol have been developed to produce low-cholesterol dairy products. These include steam stripping, molecular distillation, solvent or super-critical extraction, reaction with cyclic anhydride, enzymatic method and treatments with adsorbents like saponin, activated charcoal and cyclodextrin. These are briefly discussed below.

1. Steam Stripping

This process is similar to that used in the deodorization of vegetable oils and removal of unsaponifiable matter. To remove cholesterol by steam stripping, the fat is first deairated under vacuum after which it is heated with steam up to 232°C and then subjected to steam at low pressure in cylindrical tall chamber. The anhydrous milk fat (AMF) passing over a series of plates is spread in many thin layers, which increases the stripping efficiency. The steam rises and carries with it the evaporated cholesterol to be condensed and collected with other volatiles. This process can remove up to 93% of cholesterol though with 5% fat losses. The major disadvantage to the process is that it removes flavouring compounds also (Schlimme & Kiel, 1989).

2. Molecular Distillation

In this process, AMF is molecularly distilled at temperature 190 and 210°C at a vacuum of 10⁻⁴ Torr. Fractions distilled at 190 and 210°C represented 3.43 and 3.99% of the initial mass and contained more than 93% of the total cholesterol (Lanzani et al., 1994 and Sharma et al., 1999). Arul et al. (1988) fractionated AMF into four fractions at temperatures of 245 and 265°C and pressure of 220 and 100 mm Hg. Two low melting point fractions were blended together to yield a total of three fractions (liquid, intermediate and solid). About 78% of the total cholesterol was found in the liquid fraction while the remaining was found in the intermediate (18%) and solid (4%) fractions in the esterified form. But, because of the high heat used in the process, the quality of the end product is adversely affected.

3. Solvent Extraction

In this process butter oil is mixed with propane and ethanol in the mixing vessel. The low viscous mixture of butter fat, ethanol and propane is fed into the extraction column. A mixture of ethanol and water, containing a small amount of propane is used as extractant. The extract, a solution of cholesterol and butter fat in a mixture of ethanol, water and some propane is withdrawn at the bottom of the extraction column, which is splitted into two phases. The upper phase consists of fat and cholesterol, which are subsequently separated, in a further processing step. Around 90 to 95% of the cholesterol is extracted in this counter-current procedure operated at 30°C and 10 bar (Czech et al., 1993).

4. Supercritical Carbon Dioxide Extraction

Some studies have shown that supercritical carbon dioxide (SC-CO₂) can be used to fractionate AMF with evidence that cholesterol can be concentrated into selected fractions. Kaufmann et al., (1982) obtained two fractions of milk fat by SC-CO₂ extraction at a pressure of 200 bars and temperature of 80°C. In this process, the liquid fractions were enriched in total cholesterol. However, Huber et al. (1996) observed that direct supercritical extraction of cholesterol from AMF is not feasible because of the low selectivity of cholesterol and poor solubility of AMF. Moreover,
under these conditions, important milk flavours also get separated with the cholesterol. Therefore, they proposed another process for cholesterol removal from AMF, dissolved in SC-CO₂ under high solubility conditions for AMF (40 MPa at 70°C) to achieve rapid extraction. In this process, the dissolved AMF in SC-CO₂ is passed isobarically and isothermally through a high-pressure column, filled with a suitable adsorbent (e.g. silica gel) to eliminate cholesterol. Finally, the supercritical mixture is fractionated by either descending or ascending temperature profile in separators connected in series. Karkare and Alkio (1993) found that over 99% of cholesterol from milk fat could be removed using an SC-CO₂ extraction system equipped with a silica gel column.

5. Reaction with Cyclic Anhydride

Gu et al. (1994) developed a method for cholesterol removal from milk fat based on the reaction between the hydroxyl group of cholesterol and a cyclic anhydride such as succinic anhydride. The conversion of cholesterol into an acid derivative makes it possible to remove these from fats by extraction with aqueous alkali. Addition of acetic acid increases the rate of reaction and prevents the distillation of cyclic anhydride from reaction mixture. They removed 50% cholesterol from animal fats but along with it α-tocopherol (50%), γ- and δ-lactones also get removed.

6. Enzymatic Method

McDonald et al. (1983) have described an enzymatic process using cholesterol reductase for conversion of cholesterol to biologically inactive, e.g., non-toxic, non-absorbable products like coprosterol, which is either not or is only poorly adsorbed by the body. This approach, which is theoretically suitable for reducing the cholesterol content of milk fat, has been verified biologically at least in part, by the finding that a portion of the intestinal cholesterol is reduced to coprosterol by intestinal bacteria and subsequently eliminated.

7. Adsorption Methods

Cholesterol can be removed by its adsorption on certain material. Adsorbents, which are used to remove cholesterol, are activated charcoal, saponins and β-cyclodextrin.

Activated charcoal

Bindal et al., (1994) could remove half of the cholesterol present in milk fat through treatment of liquid fat with activated charcoal. Another activated charcoal method claimed 95% of cholesterol removal from AMF but many other compounds including yellow pigments were also removed simultaneously (Sharma et al., 1999).

Saponins

Saponins are naturally occurring plant compounds that can be used to selectively bind to cholesterol and precipitate it out. 80% and 90% cholesterol reduction in cream and anhydrous milk fat was obtained by using this method (Riccomini et al., 1990). Oh et al. (1998) found 70.5% of the cholesterol removal when milk was treated with 1.5% saponin at 45°C for 30 min. Further, addition of 0.25% celite increased cholesterol removal to 72%. However, the methods using activated charcoal or saponins are relatively non-selective and remove flavour and nutritional components also when cholesterol is removed (Lee et al., 1999; Sharma et al., 1999).

β-cyclodextrin

Beta cyclodextrin, one of the well known members of cyclodextrin family, is a cyclic oligosaccharide of seven glucose units joined ‘head to tail’ by α-1, 4 linkage and is produced by the action of enzyme cyclodextrin glycosyl transferase (CGT) on hydrolyzed starch syrup. Beta
cyclodextrin has torus like structure. The central cavity is hydrophobic, giving the molecule its affinity for non-polar molecules such as cholesterol (Szejtli, 2004). The radius of the cavity can accommodate a cholesterol molecule almost exactly, explaining the highly specific nature of β-cyclodextrin’s ability to form an inclusion complex with cholesterol (Hettinga, 1996).

References


Application of Nanotechnology in Dairy Processing

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Introduction

Nanotechnologies offer some real and wide-ranging benefits to the whole of the food chain. A number of nanotechnology-derived ingredients, additives and food contact materials are already available worldwide. But applications of nanotechnology in food sector are relatively recent compared with its use in drug delivery and pharmaceuticals. Nanotechnology has shown great potential for improving the effectiveness and efficiency of delivery of nutraceuticals and bioactive compounds in functional foods to improve human health. Bionanocomposites are hybrid nanostructured materials with improved mechanical, thermal and gas barrier properties. In addition to food packaging and nanodelivery systems of bioactive components, food preservation is also of great importance for the food industry. Food spoilages can be detected with so called nanosensors. Some of the applications nanotechnology include: improved taste, flavour, colour, texture and consistency of food stuffs, increased absorption and bioavailability of nutraceuticals and health supplements, development of food antimicrobials, new food packaging materials with improved mechanical barrier, antioxidants and antimicrobial properties, nanosensors for traceability and monitoring the condition of food during transport and storage. It can enhance solubility, facilitate controlled release, improve bioavailability, and protect the stability of micronutrients and bioactive compounds during processing, storage, and distribution. In this paper the aspects of nanotechnology that are related to food quality involving the development of health foods, food packaging materials as well as the nanosensors for the detection of microbial and chemical contaminants has been focused.

Nanoparticulate delivery systems

The current global interest in developing health-promoting foods provides a suitable opportunity to make use of bioactive components in such food formulations. Many bioactive components simply are added to functional foods in the form of pure preparations, extracts from plant or animal origin. In some cases, such nutrients are not so easily incorporated in foods because of their poor solubility; sensitive to oxygen, light, temperature, or undesirable sensory attributes. In other cases, the nutrient binds so tightly to the food matrix that it is not readily available to be taken up by the digestive system during the limited residence period in the gastrointestinal tract (GI). In such cases, new emergent approach like nanoencapsulation can be used to deliver the bioactive components. These nanocarrier systems can be used to mask the unpleasant tastes and flavours of ingredients, to protect the encapsulated ingredients from degradation during processing and storage, to improve dispersion of water-insoluble food ingredients as well as to protect food antimicrobials from interfering food components and improve their delivery to the specific site. Nanoencapsulation is currently the second largest area of nanotechnology application in the food sector which can be used as a strategy to harness a controlled delivery system for food ingredients in process food.

Nanoencapsulation is defined as a technology to pack substances in miniature making use of techniques such as nanocomposite, nanoemulsification, and nanostructuration and provides final product functionality that includes controlled release of the encapsulated bioactive components. The
Nanoliposome technology provides opportunities for food formulators in areas such as encapsulation and controlled release of food materials, as well as the enhanced bioavailability, stability, and shelf-life of sensitive ingredients. The application of nanoliposomes as carrier vehicles of nutrients, nutraceuticals, enzymes, food additives, and food antimicrobials was reported (Mozafari et al. 2008). Coenzyme Q_{10} nanoliposomes were produced with the desired encapsulation quality and stability (Mozafari et al. 2006). Liposomes, or lipid vesicles, are formed from polar lipids that are available in abundance in nature, mainly phospholipids from soy and egg. Taylor et al. (2005) reviewed food applications of liposomes to increase shelf life of dairy products by encapsulating lactoferrin, a bacteriostatic glycoprotein as well as nisin Z, an antimicrobial polypeptide.

Nanoemulsions are simply very fine oil-in-water (o/w) emulsions with mean droplet diameter of 50–200 nm. An emulsion is defined as a mixture of two completely or partially immiscible liquids, such as oil and water, with one liquid being dispersed in the other in the form of droplets. Examples of emulsified food products are mayonnaise, milk, sauces, and salad dressings. Bioavailability of lipophilic active ingredients can be substantially improved by delivery in nanoemulsions. Because of their small size, they may also exhibit some interesting textural properties that differ from those of an emulsion containing larger droplets. For example, they may behave like a viscous cream even at low oil droplet concentration; this fact has attracted attention in the development of low-fat products.

Micelles are submicron spherical particles, typically 5–100 nm in diameter, that are formed spontaneously upon dissolution of surfactants in water at concentrations that exceed a critical level, known as the “critical micelle concentration” (CMC). A remarkable property of micelles is that they have the ability to encapsulate nonpolar molecules such as lipids, flavorants, antimicrobials, antioxidants, and vitamins (Weiss and McClements, 2002). Reports of successful application of microemulsions include encapsulation of limonene, lycopene, lutein, and omega-3 fatty acids using a variety of food-grade emulsifiers, although in some cases addition of ethanol as a co-surfactant was required. Patent applications have been filed for the use of microemulsions to incorporate essential oils in flavored carbonated beverages (Weiss and McClements, 2002).
**Biopolymeric** nanoparticles consist of a matrix of biopolymers that may be linked through intermolecular attractive forces or through chemical covalent bonds to form solid particles. Globular proteins such as whey proteins have the ability to denature, dissociate, and aggregate under different conditions of pH, ionic strength, and temperature to form particles with size ranging from 40nm to 2 mm. These properties can be judiciously exploited to formulate active molecule-loaded particles of specific size. When biopolymers are combined with nanoparticles, the resulting bio-nanocomposites exhibit significant improvements in the mechanical properties, dimensional stability and solvent or gas resistance with respect to the pristine polymer due to high aspect ratio and high surface area of nanoparticles. Protein based nanoparticles can also conjugate nutrients via either primary amino groups or sulfhydryl groups. Electrostatic nanocomplexes consisting of β-Lg and pectin, which entrapped DHA molecules, showed a very good colloidal stability, and had a mean particle size of about 100 nm, resulting in transparent dispersions, potentially useful for enrichment of acid non-fat drinks, particularly clear ones. In addition, this new technology was shown to effectively confer protection to DHA against its oxidation during an accelerated shelf-life stress test (Zimet and Livney, 2009).

Understanding the mechanism of targeted delivery will enable food manufacturers to design smart food systems capable of ensuring the optimal health of each individual citizen. In particular, research on nanoemulsion will increase because of the transfer from parallel efforts in the drug delivery sector (ObservatoryNANO, 2010). However, there are some issues to commercialization for nanoemulsions. First, suitable food-grade ingredients must be identified for formulating food nanoemulsions. Second, many of the approaches that have been developed within research laboratories may not be suitable for scale-up to industrial production; suitable processing operations must be identified for economic production of food-grade nanoemulsions on an industrial scale. Third, as nanodroplets may have an enhanced bioavailability, *in vivo* evaluation of nanoemulsion droplets is required; however, such studies are limited (McClements and Jiajia, 2011).

It can be foreseen that with improvements in manufacturing technologies, new strategies for stabilization of fragile nutraceuticals and development of novel approaches to site-specific carrier targeting, food-component-based materials will play an important role in increasing the efficacy of functional foods over the next decade. However, at the present stage, nano-encapsulation still requires fundamental research to fully define the relevant parameters controlling the system. So there is need to establish methods which can be scaled up for preparation of nanoparticles such as nanoemulsions, nanoliposomes and nanomicelles etc. with special reference to encapsulate bioactive components to ensure design of ideal nutraceuticals carriers for use in the food industry. Studies are required to evaluate the stability of bioactive encapsulated nanosystems under different environment of foods (such as oxygen, light, temperature, pH and water), compatibility with food matrix and interactions with different components of food.

**Bio-nanocomposites as food packaging materials**

Food packaging like any other packaging is an external means of preservation of food during storage, transportation and distribution. Food packaging is the largest user of plastics (~40%). In India, as per the Central Pollution Control Board, approximately 15,300 tonnes of plastic waste is generated per day (The Hindu, 2011). The volume of plastics discarded annually creates a substantial waste which is causing a great threat to environment. Consequently, the approach of making materials from biodegradable materials that can be disposed of through composting or recycling got momentum. Biopolymers from agricultural food stocks, food processing waste and other resources have the ability upon blending and/or processing to result in biopolymeric packaging material called as biodegradable polymers or bioplastics (Davis and Song, 2006). Unfortunately, so far the use of biodegradable films
for food packaging has been strongly limited because of the poor barrier properties and weak mechanical properties shown by natural polymers (Kumar et al., 2011). Recently, a new class of materials represented by bio-nanocomposites has proven to be promising option in improving the mechanical, barrier and thermal properties of these biopolymer-based packaging materials. Polymers can also be added with suitable fillers to form composites for enhanced barrier properties.

**Polymer nanocomposites** are created by dispersing an inert, nanoscale filler throughout a polymeric matrix in which the filler has at least one dimension smaller than 100 nm. Filler materials could be either flakes, fibers, whiskers or nanoparticles. The mechanical, thermal and barrier of nano-composites are often remarkably different from those of non-reinforced biopolymer-based materials. Addition of relatively low levels of nanoparticles (less than 5%) have been shown to substantially improve the properties of finished plastic, increasing the deformability and strength, and reducing the electrical conductivity and gas permeability. Further, polymers when incorporated with certain nanoparticles have the ability to interact with the food/environment and package and confer antimicrobial properties and while some continuously monitor and detect changes in the package environment.

Various inorganic nanoparticles have been recognized as possible additives to enhance the polymer performance. Among all, as of now the layered inorganic solids like clay have attracted attention by packaging industry. This is not only due to their availability and low cost but also due to their significant enhancements and relatively simple processability. Montmorillonite (MMT), hectorite, saponite and kaolinite are the commonly used layered silicates (Ray and Okamoto, 2003; Duncan, 2011). These structural characteristics contribute to MMT’s excellent utility as a filler material for polymer nanocomposites, typically giving rise to impressive increase in polymer strength and barrier properties.

Starch-clay is the most often cited biodegradable nanocomposites investigated for various applications including food packaging significant improvements in mechanical properties were reported with the addition of montmorillonite (MMT) clay. Azeredo et al. (2009) incorporated cellulose nanofibers in mango puree films to improve functional properties viz. tensile properties, water vapour permeability and glass transition temperature. Das et al. (2011) studied the physico-chemical properties of the jute micro/nanofibril reinforced starch/polyvinyl alcohol biocomposite films. Yu et al. (2006) reported that through the use of high-powered ultrasonics, soy protein/clay nanocomposites could be produced, which exhibited improvement in modulus. Kumaret al. (2010) studied the effects of the pH of film forming solution, MMT content, and extrusion processing parameters on the structure and properties of soy protein isolate-MMT bio-nanocomposite films.

**Bioactive Packaging**, in addition to proving as a passive barrier, packaging can contribute to the control of microbial growth in food products, which cause spoilage or in case of pathogens, diseases and illness. Antimicrobial (nisin, silver oxide, zinc oxide, magnesium oxide) nanoparticle and antioxidant coatings in the matrix of the packaging material can reduce the development of bacteria on or near the food product, inhibiting the microbial growth on non-sterilized foods, maintain sterility of pasteurized foods by preventing post-contamination and prevent oxidative changes in food thereby improving the quality. Most antimicrobial activities of nanocomposites have centered on nanoparticles of silver and zinc oxide. Silver has a long history of being used as an antimicrobial agent in food and beverage storage applications. Cellulose is a good carrier of silver nanoparticles. Fernandez et al. (2010) developed cellulose-silver nanoparticle hybrid materials (cellulose-based silver loaded absorbent pads) and demonstrated that when fresh cut “Piel de Sapo” melon was placed over the pads, the pads released silver ions after the melon juice impregnated the pad and controlled the spoilage-related bacteria.
Titanium dioxide (TiO$_2$) coated packaging film has shown to considerably reduce *E. coli* contamination on food surfaces. TiO$_2$ is a photocatalytic disinfecting material for surface coatings (Chawengkijwanich and Hayata, 2008). Recently, a starch/ZnO-carboxymethylcellulose sodium nanocomposite was prepared using ZnO nanoparticles stabilized by carboxymethylcellulose sodium (CMC) as the filler in glycerol plasticized-pea starch (Yu et al., 2009). Busolo et al. (2010) developed novel silver-based nanoclay as an antimicrobial in PLA food packaging coatings.

Further bioactive packaging materials need to be able to keep bioactive compounds, such as prebiotics, probiotics, encapsulated vitamins or bioavailable flavonoids, in optimum condition until they are released in a controllable manner into the food product (Lopez-Rubio, et al. 2006). Bioactive-packaging materials can help to control oxidation of food stuffs and to prevent the formation of off-flavors and undesirable textures of food. Bioactive compounds that are encapsulated into the packaging itself are a promising approach because this would allow the release of the active compounds in a controllable manner. Several already approved food additives could be used for such nanoencapsulation, including carrageenan, chitosan, gelatin, polylactic acid, polyglycolic acid and alginate (Lopez-Rubio et al., 2006).

**Smart packaging** is a package that continuously monitors the internal environment and responds or communicates the changes to external environment and/ or consumer, beyond performing the basic functions, is called as a smart package and the technique as smart packaging. The unique chemical and electro-optical properties of nanoscale particles enable them to be part of a smart package. Such nanoparticles can be used to detect the presence of gases, aromas, chemical contaminants and pathogens. Mills (2005) reported development of a promising photoactivated indicator ink for in-package oxygen detection based on nanosized TiO$_2$ or SnO$_2$ particles and a redox-active dye (methylene blue).

With the worldwide growing scientific evidence of the potential benefits of nanotechnology in food packaging, there exists huge scope for us to get benefitted in India as such applications are at nascent stage. Biopolymers from agricultural food stocks, food processing waste and other resources could be exploited for developing nanocomposite films for packaging applications.

**Application of nanotechnology in analytical chemistry**

Food spoilages can be detected with so-called nanosensors, for example, an array of thousands of nanoparticles designed to fluoresce in different colors on contact with food pathogens. Taking into account the crucial importance of time in food microbiology, the main aim of nanosensors is to reduce the time for pathogen detection (Bhattacharya, et al. (2007). Such nanosensors could be placed directly into the packaging material, where they can detect chemicals released during food spoilage. Other types of nanosensors are based on microfluidics devices (Baemummer, A. 2004) and can also be used to detect pathogens efficiently in real time and with high sensitivity.

In the food-analysis market, devices produced with the so-called nanoelectromechanical systems (NEMS) technology are already in use and these systems contain moving parts ranging from nano- to milli-meter scale, which might serve as developing tools in food preservation. They can control the storage environment and act as active ‘sell by’ devices. NEMS could be used in food quality-control devices because they consist of advanced transducers for specific detection of chemical and biochemical signals. The use of so-called micro- and nano-technologies (MNTs) have several advantages for food technology, such as portable instrumentation with quick response, low costs and smart communication through various frequency levels. In the area of food safety and quality, MNTs are particularly suitable because they are able to detect and monitor any adulteration in packaging and storage conditions (Canel, et al. 2006). Nanocantilevers are another innovative class of biosensors.
Their detection principle is based on their ability to detect biological-binding interactions, such as between antigen and antibody, enzyme and substrate or cofactor and receptor and ligand, through physical and/or electromechanical signaling (Hall, R. H., 2002). They consist of tiny pieces of silicon-based materials that have the capability of recognizing proteins and detecting pathogenic bacteria and viruses (Kumar, C.S.S.R. 2006). Nanocantilever devices have already had tremendous success in studies of molecular interactions and in the detection of contaminant chemicals, toxins and antibiotic residues in food products (Ramirez Frometa, N. 2006). Pathogen detection is based on their ability to vibrate at various frequencies in dependence on the biomass of the pathogenic organisms. The silicon surface of nanocantilevers can be modified to attach antibodies, resulting in a change of the resonant frequency depending on the attached mass. Gfeller et al. (2005) were able to detect Escherichia coli, which is an indicator of fecal pollution of water and food products, with the help of a cantilever coated with agarose.

Biggest application of nanotechnology in the area of analytical chemistry and diagnostics is the development of lateral flow based assays. The development of lateral-flow assays has provided a convenient and inexpensive means for identification of target substances in biological specimens and a well known example is the commercially available pregnancy test kit. These test strips and devices have found numerous applications in testing of drugs of abuse, infectious diseases, and pregnancy, just to name a few. They are easy to store as the reagents are provided in a dry or nearly dry state. Components of lateral flow assay include nitrocellulose membrane, fiberglass pads and absorbent pads. In such flow system, usually gold particles of nanometer size are used. Gold nanoparticles (GNP) exhibits flow properties and therefore it is advantageous to label GNP with ligand for their recognition by target molecules. Since GNP are coloured, exhibits flow characteristics and can be functionalized for attachment of ligands; GNP are widely used in Lateral Flow Assays. The movement of GNP through pores of membrane is fast and therefore results can be obtained in minutes. Specificity of ligand towards target molecules determines reliability of results.

Later on in 2006, Liu et al., have demonstrated the aptamer-based lateral flow devices for the detection of adenosine and cocrain in pure solutions and in serum. Aptamers are single stranded DNA or RNA molecule of about 20-100 nucleotides which can recognize target molecule. Identification of sequences of nucleotides for selecting specific aptamer is tedious process. Aptamers can bind GNP and can also be labeled with biotin; these can be used as ligands in lateral flow system. The interaction of aptamers with targets can be measured and therefore, specific aptamers can be selected. Over the year a number of publications have appeared in this area and concept is being extended for the detection of contaminants, adulterants and pathogen detection in food system. In near future, lateral flow system involving gold nanoparticles and aptamers will be very common for detection of analyte.

**Regulatory Frameworks**

Nanomaterials which include nanoparticles, nano-emulsions and nano-capsules are now being used in processed foods. The market penetration of such products in different countries will depend on, amongst other factors, the price and quality of the products. Uncertainty exists over the regulations of nano-based products and which linked in part with the lack of necessary safety data. So this indicates the urgent need of toxicological studies and safety evaluation. It means that there will be a growing need for strategies to regulate the risks, and establishment of liabilities, at the global level. This will inevitably pose a bigger challenge for the regulatory authorities because food laws in different countries may not conform to each other. In due course, such issues are likely to be resolved through the development of global frameworks that relate to key international trade agreements, such as those administered by the World Trade Organization (Hodge, 2007). The Chemical Selection Working
Group of the U.S. Food and Drug Administration (FDA) defined nanomaterials as “particles with dimensions less than micrometer scale (i.e. less than 1,000 nm) that exhibit unique properties not recognized in micron or larger sized particles” (U.S. FDA 2006). Australia’s Commonwealth Scientific and Industrial Research Organisation (CSIRO) food scientists have also defined nanomaterials as measuring up to 1,000 nm (Sanguansri and Augustin, 2006). In another report on nanomaterials FDA chose not to offer a size-based definition at all (U.S. FDA, 2007). Whether a product would be considered to be a nanomaterial or representing an application of nanotechnology also depends on available definitions applied by regulators. Several regulatory bodies have meanwhile introduced or proposed definitions of nanomaterials for regulatory purposes that reflect one of the two main issues of the discussion: whether the dimension of materials of nanometer scale or the change of the properties of materials due to smaller particle size is more relevant. One definition extends the possible range of materials of concern to dimensions that are 10 times higher than the nanoscale range of 1–100 nm that was defined by the 2009 expert meeting of FAO/WHO. There is a trend to apply in the definitions two criteria, an altered or new dimension at nanoscale and a concurrent change of properties due to the change of dimension. It is evident from recent regulatory reviews that, at present, there is no nano-specific regulation anywhere in the world. Furthermore, there is a lack of specific guidelines, guidance documents for testing, or testing requirements under any of the existing regulations that relate specifically to nanoparticles in terms of size or other distinct physicochemical properties. Studies have, however, regarded the existing models for risk assessment applicable to materials and products of nanotechnology, highlighting the need for certain modifications in testing methodologies. There are also major knowledge gaps in relation to the effects of most nanoparticles on human health, agreed dose units for hazard and exposure assessments, and reliable and validated methods for measurement and characterisation of nanoparticles in complex food matrices. Studies carried out to identify potential regulatory gaps (Chaudhry et al. 2006, 2007 and 2008) have highlighted certain uncertainties and inadequacies in the existing regulatory frameworks in relation to the use of nanotechnologies in food.

However, such technological developments are still new or emergent in India and many other countries, where there is only a marginal level of current applications. Considering the global nature of food business, and that several companies and research institutions are currently exploring new possible applications in the food and related sectors, it is not unreasonable to expect that nanofood products will be available to the consumer in an increasing number and variety in the coming years. Nanotechnology applications for food and health food sectors have undoubtedly opened up enormous opportunities for innovation and new developments, but at the same time have also raised new challenges in regard to ensuring the consumer safety.

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Fortification of Milk with Mineral and Vitamins

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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

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 fortifications 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

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.

**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).

**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.

**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.

**References**


New Approaches to Ascertain the Quality of Ghee

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Introduction

Milk fat is an important component in the dairy industry, which plays a significant role in economic, nutrition, physical and chemical properties of milk and milk products. Milk fat is one of the valuable fats that continue to be a target of unscrupulous traders for the maximization of profits. Methods presently adopted by food law enforcing agencies to ensure the quality of milk fat are mainly based on the physico-chemical constants like [Butyro- refractometer reading (B.R – reading), Reichert-Meissl value (R.M – value) and Polenske Value (P. value) Phyto- sterol Acetate test (PA- test) and Baudouin- test]. However, all these methods fail when milk fat is adulterated with a mixture of body fats and vegetable oils. In addition to this, now days tailored vegetable oils with R.M/ P.V and B.R close to that of milk fat are available to the unscrupulous people in the unspecified market for adulteration purposes. Researchers have tried some innovative ideas to counter this menace of adulteration. The innovative approaches include change in the ratios of different fatty acids, sterol analysis, and Carbon number profiling of Triglycerides and coupling of fractionation with other parameters. In the present article an attempt has been made to compile the information on some recent approaches developed to detect the adulteration of milk fat. Some of the described approaches are being used by International testing agencies, but needs validation in Indian situation.

Apparent solidification time (AST) test.

The apparent solidification time (AST) of the fat sample is defined as the time taken by the melted fat sample to get solidified apparently at a particular temperature. The test can be carried out as:

Take 3.0 g of completely melted fat sample in a test tube (10 × 1.0 cm ID) and maintain at 60°C for 5 min. Transfer the test tube in a refrigerated water bath maintained at 18 ± 0.2°C and simultaneously start the stop watch. Observe the test tube constantly till the apparent solidification of the fat sample takes place which is confirmed by non-movement of fat sample on tilting the test tube. At this stage stop the stopwatch and record the time taken for the apparent solidification of the fat. Pure ghee sample of both cow and buffalo shows AST in the range of 2 min 31 s to 3 min 25 s. Any deviation from these values gives an indication of adulteration of milk fat (Kumar, 2009).

AST coupled with dry fractionation technique

By employing dry fractionation technique, the different fractions enriched with body fats or vegetable oils are obtained and subsequently used to estimate AST. The aim is to enrich the solid fraction with animal body fats and liquid fraction with vegetable oils. Vanaspati, if added, will also be fractionated along with animal body fats.

Take 100 g of clarified melted fat and keep it in a BOD incubator maintained at 20 ± 0.1°C. After about 1.50 to 1.75 h of incubation, approximately one third of the whole fat gets solidified. Separate the solid fraction (S20) from the remaining liquid portion by filtration inside a BOD incubator maintained at 20 ± 0.1°C. Further fractionate the liquid portion thus obtained in another BOD incubator maintained at 18 ± 0.1°C for 2 h so as to obtain another solid (S18) and liquid (L18) fraction by filtering inside a BOD incubator maintained at 18 ± 0.1°C. Analyse S20, S18 and L18 fractions of ghee for AST as described above. S20, S18 and L18 fractions of pure ghee of both cow and
buffalo show AST values of 1 min 40 s to 2 min 50 s; 2 min 30 s to 3 min 40 s and 2 min 50 s to 3 min 50 s, respectively. Any deviation from these values gives an indication of adulteration (Kumar, 2003).

**Complete liquification time (CLT) test**

The complete liquification time (CLT) test of the fat sample is defined as the time taken by the solidified fat sample to get melted completely at a particular temperature. The test can be performed, as follows:

Take 3.0 g of completely melted fat sample in a test tube (10 × 1.2 cm) and maintain at 60°C for 5 min. Keep the test tube containing fat sample in a refrigerator (6- 8°C) for 45 min for solidification of the melted fat sample. Transfer the test tube in a water bath maintained at 44 ± 0.1°C and simultaneously start the stop watch. Observe the test tube constantly till the fat sample is completely liquefied. At this stage stop the stopwatch and record the time taken for complete liquefication of the fat. Pure ghee sample of both cow and buffalo shows CLT in the range of 2 min 12 s to 3 min 15 s. Any deviation from these values gives an indication of adulteration of milk fat (Kumar, 2008).

**Complete liquification time (CLT) test coupled with solvent fractionation technique**

Using solvent fractionation technique, the different fractions enriched with body fats or vegetable oils can be obtained and used subsequently to estimate CLT. Here also, the aim is to concentrate animal body fats into solid fraction and vegetable oils into liquid fraction. Vanaspati, if added, will also be concentrated in solid fraction along with animal body fats.

Take 30 g of melted ghee sample in a 100 ml graduated glass tube, and then add 60 ml acetone and mix well to dissolve the fat. After mixing, keep the sample at 40°C for equilibration for 5 min. Then subject the sample in a refrigerated water bath to three temperatures/time combinations, viz., 16 ± 0.1°C/25 min, 8 ± 0.1°C/25 min and 4 ± 0.1°C/60 min, successively, after filtration at each stage of time/temperature combination. After about 25 min at 16 ± 0.1°C, approximately one-fourth of the whole fat gets solidified. This first solid fraction (S₁₆) obtained at 16 ± 0.1°C is separated from the remaining liquid portion (L₁₆) of the whole fat by filtration through ordinary filter paper. The remaining liquid portion (L₁₆) thus obtained after filtration is further fractionated at 8 ± 0.1°C in refrigerated water bath. After about 25 min, it gets partitioned into two fractions, one solid (S₈) and one liquid (L₈), which can be separated by filtration through ordinary filter paper. At last, L₈ fraction is further fractionated at 4 ± 0.1°C for 60 min and filtered to get two fractions, one solid (S₄) and one liquid (L₄). Finally at the end of fractionation, three solid fractions (S₁₆, S₈ and S₄) and one liquid fraction (L₄) are obtained from ghee sample containing a mixture of adulterants. Solvent from liquid fraction is removed by using rotary evaporator at about 40°C, followed by nitrogen flushing to evaporate solvent completely from the liquid fraction. To get rid of entrapped acetone, respective solid fractions are heated to 110°C for about 2 h in an oven.

(a) **Analysis of first fraction (S₁₆) for CLT at 46°C**

Analyze S₁₆ fraction for CLT at 46 ± 0.1°C (instead of 44 ± 0.1°C used for CLT of whole fat) as described above. CLT values of S₁₆ fraction at 46°C range between 4 min 5 s to 9 min for both cow and buffalo pure ghee. Any deviation from these values gives an indication of adulteration of milk fat (Kumar, 2008).

(b) **Analysis of last fraction (L₄) for Iodine value**

Analyze L₄ fraction for iodine value as described above. The iodine values for L₄ fraction of pure cow and buffalo ghee are found to vary between 37.85- 46.48. Any deviation from these values gives an indication of adulteration of milk fat (Kumar, 2008).
Modified Holde’s test for liquid paraffin in ghee
Isolate the fat from milk by heat clarification method as described above. Saponify 1 g of fat taken in a test tube with 5 ml of 0.5 N ethanolic KOH solutions by heating on direct flame, using wire gauge for 5 min. Add about 5 ml of distilled water to the hot saponified solution. Appearance of turbidity indicates the presence of mineral oil (Kumar, 2005).

Rapid color based test for detection of vegetable oils
One ml of clear molten fat was dissolved with 1.5 ml of hexane in a tightly capped test tube. To this was added 1.0 ml of color developing reagent (distilled water, Sulphuric acid - Sp.gr.1.835 and Nitric acid- Sp. gr. 1.42 in the ratio of 20:6:14), shaken vigorously and kept undisturbed till it is separated into two layers. The appearance of a distinct orange tinge in the upper layer indicates the presence of vegetable oils / fats including vanaspati (Sharma et al., 2007).

Temperature controlled attenuated total reflectance- mid- infrared (ATR-MIR)spectroscopy:
This is a spectroscopic technique used for the rapid estimation of butter adulteration. Themethodology is typically based on the infra-red spectroscopic technique (Koca et al., 2010). These workers collected the Fourier transform infrared spectra of the samples between 4000 and 650 cm⁻¹ on a FTIR spectrophotometer. Here the temperature was controlled, which allowed the stabilization of analysis temperature at 65± 2°C. The data was analyzed by using statistical tool namely Multivariate analysis and calibration models were developed covering all possible adulteration ratios. In this case adulteration of butter with margarine @ 2.5% could be predicted.

FTIR Spectroscopy
The adulteration of butter fat with foreign fat could be detected by observing the FTIR spectra at the specific wavelength due to the ratios of cis-unsaturations of fatty acid moieties as reported by Sato et al., (1990). Nurrulhidaiyah et al., (2013) employed FTIR coupled with chemometrics and PLS analysis and successfully detected adulteration of milk fat with beef fat and chicken fat at the frequency region of 1500-1000 cm⁻¹ (beef fat adulteration) and 1200-1000 cm⁻¹ (chicken fat adulteration), respectively.

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS)
Mass spectrometry is being increasingly used to identify adulterations in food and milk due to its simplicity and the need for minimum sample preparation (Sanvido et al., 2010). Matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-QTOF MS) is a special type of M.S which demonstrated the information on the basis of m/z (m – mass of a molecule, z- charge of a molecule) with the help of special matrix, was seen to be a valuable technique for the analysis of oils and fats by providing characteristic profiles of triglycerides. MALDI-QTOF MS provided rapid and unambiguous profiles of the TAG composition, and that the TAG are detected mostly as sodiated molecules [TAG+Na]+ (Saraia et al., 2009). They reported that in case of pure milk fat the ions of m/z 881.8, 907.8, 909.8 and 911.8 corresponds to [TAG+Na]+ with the POO, OOO, OSO and SSO compositions (P, palmitic acid; O, oleic acid and S, stearic acid), respectively, which are changed during adulteration with any foreign fat. Garcia et al., (2011) reported that during adulteration of milk fat with vegetable oil MALDI-QTOF MS spectra data changed due to change in the tri acyl glycerol characteristic, this phenomena due to the illegal addition of non-milk fat to milk is shown to mostly increase the relative abundance of the ion clusters centred on charge by mass ratio of triacylglycerol.
Tests based on gas liquid chromatography of triglycerides

European Union has selected this methodology as official reference method to detect non-milk fat in milk fat by T-G analysis using packed or capillary column and low-resolution gas chromatography (GC). International Organization for Standardization (ISO) and International Dairy Federation (IDF) jointly specify a method to detect non-milk fat adulterants in milk fats based on TG compositions and in combination with standardised formulae known as standardised (S) values (STotal, S2, S3, S4 and S5) for different kinds of adulterants (ISO 2010). Recently, this approach has been used by Kalla Amrutha (2013), in detecting the possible adulteration of market samples of ghee. Author has performed the Triglyceride (TG) analysis using low-resolution Gas chromatography wherein nitrogen was used as carrier gas. HP-5, capillary column of 2.5 m length (cut from 15 m 9 0.25 mm 9 0.25 lm), column flow 1.20 mL/min of nitrogen carrier gas, with a split ratio of 1:10. The chromatographic conditions were as follows: the initial oven temperature of 200 °C was increased to 325 °C at the rate of 5 °C/min and held at the final temperature for 10 min. The injector and detector temperatures were 330 and 360 °C, respectively. For TG analysis, 1 µl of 5 mg/mL of control and commercial ghee samples prepared in hexane and Supelco TG standard mix (5 TG mix- 100 mg neat mixture of 99% pure 20% each of C8:0, C10:0, C12:0, C14:0 and C16:0 acids) from Bellefonte, PA, USA, was injected to GC. ISO/IDF specified equations as mentioned below were used to find out the level of adulteration in market samples.

<table>
<thead>
<tr>
<th>S value</th>
<th>Field of application</th>
<th>ISO/IDF specified equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2 (98.05-101.95)</td>
<td>Soya bean, Sunflower, olive, Rape-seed, Linseed, Wheat germ, maize germ, Cotton seed &amp; Fish oil</td>
<td>2.083SC30 + 0.728SC34 + 0.692SC36 + 0.635SC38 + 3.745SC40 - 1.292SC42 + 1.354SC44 + 1.701SC46 + 2.528SC50</td>
</tr>
<tr>
<td>S3 (99.42-100.58)</td>
<td>Coconut &amp; Palm kernel fat</td>
<td>3.745SC52 + 1.113SC54 + 1.364SC58 + 2.154SC62 + 0.427SC64 + 0.580SC66 + 1.292SC68 + 1.090SC70 + 0.995SC72 + 1.255SC74</td>
</tr>
<tr>
<td>S5 (97.96-102.04)</td>
<td>Lard</td>
<td>6.512SC66 + 1.205SC70 + 1.736SC74 + 1.755SC76 + 2.233SC78 + 2.806SC80 + 2.543SC82 + 0.989SC84</td>
</tr>
</tbody>
</table>

Reversed phase thin layer chromatographic method:

Recently, a reversed-phase thin layer chromatographic protocol has been developed by Anupma; 2013. The method is based on the detection of tracer component i.e. β-sitosterol. The presence of β-sitosterol band in addition to cholesterol band on chromatographic plate indicates the presence of vegetable oils in ghee. In this method unsaponifiable matter is extracted from 0.2 g fat using 5 ml of 5% methanolic KOH in a screw capped test tube. The tube is incubated in a water bath maintained at 90°C with intermittent shaking after every 5 minutes, for about 20 min. After 20 min of incubation the tube is cooled to room temperature under tap water. One ml water and 5 ml hexane are added in the tube and tube is vortexed for 1-2 minute followed by centrifugation at 2000 rpm for about 2 minutes.
The upper hexane layer is pipetted out and dried. The unsaponifiable matter is dissolved in 500 µl of chloroform. 6 µl of the unsaponifiable matter solution is spotted on TLC silica gel 60 RP-18 F 254 plate at a distance of about 1 cm from the bottom. Plate is developed in a solvent consisting of Petroleum ether: Methanol (20:40:40 v/v). Color is developed on the plate by spraying phosphomolybdic acid solution (20% solution in ethanol) and keeping the plate at 90 - 95°C / 3 min. In adulterated samples additional band corresponding to β-sitosterol appears, whereas in pure ghee only one band corresponding to cholesterol is seen.

References:
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Microstructure of Traditional Dairy Products

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Introduction
The use of microscopy to study foods has gained increasing popularity over recent years, as it has become recognized as an essential technique that can provide a link in understanding why food products have as an essential technique that do. Generally the use of microscopy in food applications can be divided into three main areas:

- To identify foreign bodies
- To control and understand existing products or processes
- To develop new products or new processing technology

Electron microscopy was applied to dairy research for the first time by Nitschmann (1949) who studies casein micelles in cow milk. Science then milk and dairy products have been investigated by this technique rather more extensively and systematically than other human foods. During various technological treatments, the microparticulate constituent i.e. the fat globules, the colloidal casein micelles and the molecular dispersion of whey protein, undergo significant physical changes and mutual intractions. This give rise to the characteristic macroscopic structure and physical properties of the products. The ability of casein micelles to interact with whey proteins, to aggregate and hydrolyze under the influence of low pH, high heat and presence of proteolytic enzymes is crucial for preparation of dairy products. Therefore, electron microscopic of dairy products is extremely useful in elucidating the relationship between macroscopic properties and sub microscopic structure as altered by technological treatments traditional dairy products are either heat desiccated (khoa, gulab jamun) or acid coagulated or their derivatives obtained by several process modification (channa, pannear, rasogolla). The chemical composition of these products including various eddittives such as sugar and starch and the process to which they are subjected during processing determined the texture and microstructure of the products. Microstructure in turn, controls some of the attributes of the product such as elasticity, sponginess, brittleness, and firmness.

Electron microscope as a tool
The microscope has been conventionally used for the study of both biological and non biological systems. In biological sciences, it has been employed to study the structure, shape and size of specimens and also to detect contaminants. This amazing analytical tool can be put to use in food sciences beyond this basic applications. It can be used to decipher what happens to the food material such as grain, muscles or milk when they are processed in to a pasta, ham or streak, yoghurt for cheese. Food scientist can ascertain what makes a particular food to be elastic or spreadable (exp. String cheese and processed cheese) or foods which exhibit smooth texture under normal conditions become greasy (exp. Ice cream stored for long time). The optical microscope can not only be use to study structural details such as globules or fiber but also to distinguish protein from fat, starch, cellulose, mineral component and bacteria from bacteria.
Electron microscope provides a markedly higher magnification at a considerable better resolution than light microscope. Electron microscopy takes advantage of the wave nature of rapidly moving electrons where visible light has wavelength 4000-7000 Angstroms, electron accelerated to 10,000 KeV have a wavelength of 0.12 Angstroms. Optical microscopes have their resolution by the diffraction of light to about 1000 diameters magnification. Electron microscope, so far, are limited to magnification of around 1,000,000 diameters primarily because of spherical and chromatic aberrations.

**Types of electron microscopic**

There are two major electron microscopic modes- scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The electron beam is focused using magnetic lenses in both kind of microscope. The specimen is placed into the part of electron beam in the TEM but in the SEM, it is placed at the end of electron beam part. The image is produced in the form of shadow of the florescent screen in TEM where as in SEM reflected and scendory electron are process by an electron dector to form a three dimentional image on a monitor screen.

Since the electrons would be easily absorbed by air, the microscopic examination is carried out in vacuo. To ensure that the electrons will penetrate a thin section of the specimen or its replica, the electron beam is accelerated in the microscope. An anode with an orifice in its centre is positively charged. The negative electrons rush toward it and those which are in the centre fly, accelerated, through the orifice toward the specimen. Accelerating voltage of 3 to 20 kV has been used to do SEM and 60 to 80 kV have been used in TEM of foods.

Traditional electron microscopy requires that the specimen must not release any gas or vapour when inserted into the transmission or scanning electron microscope. Except for powdered foods such as flour, sugar, or milk powder, most foods contain water. Drying or freezing at a very low temperature of -100°C ensure that the condition of not releasing gas or vapour is met.

**Scanning Electron Microscopy**

The conventional method of sample preparation for scanning electron microscopy (SEM) includes chemical fixation (Glutaraldehyde, Osmic Acid), dehydration with a graded series of ethanol or acetone and subsequently drying by air drying, freeze-drying or critical point drying. The specimen is mounted on an aluminum stub and coated with heavy metal to make it electrically conductive. It has been demonstrated that simple air-drying of the specimen yields collapsed micelles even after proper fixation due to the strong interfacial forces created as a result of passage of receding water surfaces over the particles. Better results have been obtained with freeze-drying and critical point drying.

The specimen is examined by a focused electron beam. An electron gun is the source for this beam. Electrons are emitted from a cathode, accelerated by passage through electrical fields and focused to a first optical image of the source. The gun consists of tungsten or lanthanum hexaboride electrode surrounded by a shield with a circular aperture. Electrons in the gun are accelerated across a potential difference of the order of 10,000 volts between the cathode (at high negative potential) and anode (at ground potential). Some of these electrons are reflected and others generate secondary electron from the gold coating. (A great variety of other interactions also take place). Secondary electrons (or, in other applications, backscattered electrons) are used to form an enlarged image of the specimen surface. The incident electrons carry a negative charge and in order to be 'neutralized' after they have completed the examination, the specimen should be electrically conductive. As mentioned earlier this is achieved either by chemical procedures which impregnate the specimen with osmium or, more frequently, by physically coating its with gold, a gold-palladium, platinum, or iridium - occasionally both procedures are combined. Metal coating provides a path for the electrons. It this path is
interrupted (by incomplete metal coating or by cracks), the electrons sit in the area thus isolated and repel any electrons in the incidental beam in accordance with the rule that electrically charged particles of the same charge repel each other. Thus the area occupied by the stationary negative charge is by-passed and cannot be examined. White spots or lines develop in such places and the image is characterized as suffering from charging artifacts.

**Transmission Electron Microscopy**

TEMs are patterned essentially after TLM and yield information on the size, shape and arrangement of particles which make up the specimen as well as their relationship to each other on the scale of atomic diameters. The electromagnetic lenses (first & second) determines the spot size of the electron beam generated by electron gun and also alters the spot to a pinpoint beam. Further condensor lens restricts the beam by knocking out high angles electrons and beam strikes the specimen and parts of it are transmitted. The transmitted portion is focused by the objective lens into an image which is passed down the column through the intermediate and projector lenses, being enlarged all the way.

One of the most widespread techniques of specimen preparation for electron microscopy is thin sectioning of plastic-embedded samples. This technique comprises a fixation, dehydration and finally impregnation in some suitable plastic monomer such as araldite or epon. After hardening thin sections (15 to 90 nm thick) are cut with ultramicrotome and picked up on an electron opaque metal grid of 200-400 mesh (lines/in) to provide mechanical support. Most EM grids are made of copper because it is non-ferromagnetic and thus minimally distorts the magnetic field of the objective lens. Even so, it is usual practice in high resolution studies to avoid recording images of specimens which lie close to the grid bars. The specimens are post stained with heavy metals such as lead citrate and uranyl acetate and placed into the path of electron beam.

**Microstructure of traditional dairy products**

Electron Microscopy has been employed in a number of studies to observe and characterize the microstructure of Traditional Dairy products viz paneer, chhana, khoa, sweetmeats prepared from them e.g. rasogolla, gulabjamun, burfi and kalakand, and fermented milk product e.g. Dahi.

**Chhana & Paneer**

Some cheeses (Indian Paneer cheese, South American Queso Blanco cheese, American White cheese, North American Ricotta cheese) are made by coagulating hot milk with an acid and separating the curd from the whey.

These cheeses have several features in common: the milk is first heated to at least 85°C and then is coagulated using an acid such as citric, lactic, acetic, or hydrochloric acid (or an acid precursor such as glucono-d-lactone) to a final pH value of 5.5. This means that the curd is not too acidic. The coagulated milk is then cooled and the whey is separated. The microstructure of the casein particles has a characteristic 'core-and-shell' structure (micrograph at left). (The whey contains very little whey proteins since they coagulated due to the heating and became part of the curd).

How the microstructure develops has not yet been fully explained but it is known that three essential conditions must be met: The milk must be coagulated at a temperature higher than 85°C so that whey proteins may interact with the k-casein; Whey proteins and the milk salt system must be present in the milk; The final pH value must be 5.5 ± 0.1 (Kalab et al., 1988).

As revealed by SEM, microstructure of chhana has been found to be coalesced large masses of casein whey protein complexes, interlinked with some thick bridging material interrupted by closely
interspersed small voids. Buffalo milk chhana yields structure close to cottage cheese or paneer and is characterized by denser and coarser matrix than that of cow milk chhana (Adhikari, 1992).

**Rasogolla**

*Rasogolla* has typical microstructure with least resemblance to other dairy products. Cooking severely affects the compactness of *Chhana* matrix and yields loose porous structure having large void spaces throughout the matrix. Cow milk *rasogolla* has been reported to be containing thick filamentous structure of agglomerated protein bodies arranged in folds with numerous voids in between whereas densely fused large conglomerates of protein forming layer and scale were found to characterize microstructure of buffalo milk *rasogolla*. In case of mixed milk *rasogolla*, both filamentous and scale type protein bodies prevailed in the structure. *Rasogolla* prepared from lactic acid coagulated *chhana* have been observed to be more compact and ragged protein matrix to that of *rasogolla* made from citric acid coagulated *chhana* (Adhikari, 1992, Munjal, 1993).

**Khoa**

The SEM study of heat induced structural changes taking place during the manufacture of *khoa* as well as *gulabjamun* (Adhikari, 1992) revealed that constant boiling of milk during *khoa* making led to gradual coalescence of the casein whey protein complexes to a fuzzy-agglomerated mass precipitating out as large interlinked gritty particles. Further heating effected the compaction of protein lactose complexes and Buffalo milk *khoa* exhibited more open structure than that of cow milk one while mixed milk *khoa* was slightly denser to that of buffalo milk product.

**Gulabjamun**

Frying of *khoa* led to a different structural manifestation in *gulabjamun*. The compact protein bodies of *khoa* turned to a loose matrix and the starch and associated particles interspersed loosely among them. Cow and mixed milk *gulabjamun* had comparatively denser matrix to that of buffalo milk *gulabjamun*. A comparison of microstructure of market and laboratory made *gulabjamun* revealed thread or net type structure and rosette structure respectively (Adhikari, 1992).

**Kalakand and Burfi**

The SEM studies (Kumar, 2006) of fresh *kalakand* with sucrose (control) and *kalakand* sweetened with saccharin, showed that resulting microstructure of control was a spongy 3-D structure due to a networking of casein micelles alternating with very small pockets. *Kalakand* manufactured using saccharin lacked a well defined 3-D microstructure. The microstructure of protein matrix differs from the matrix in control. The spongy texture was replaced with a loosely held structure with fewer pockets of larger size as compared to control. Hence, it is clearly evident that sucrose is responsible for maintaining the structure of *kalakand* (control) due to its water binding ability and thus providing enhanced stability. This property is lost in *kalakand* manufactured using alternative sweetener saccharin.

A similar pattern in the formation of microstructure was evident in control and *burfi* made with succharin. The control *burfi* had a well defined compact globular structure while replacement of sucrose with saccharin resulted in development of microstructure which had lost most of its globular nature. The microstructure appeared to be open or loosely held. This difference can be ascribed to the presence of sugar in the control samples.

The compactness of the network decreased with the use of alternate sugar (saccharin). The loose network in these samples may explain the low hardness, cohesiveness and accordingly gumminess.
and chewiness in these samples. Hence, each of the two systems resulted in a distinct type of microstructure in the finished product i.e. burfi and kalakand.

**Dahi**

A microstructural comparison (Gupta et al., 2000) of market sample and laboratory made mishti Doi revealed that former had ill defined microstructure with yeasts present as contaminants while the latter was shown to have a clear protein matrix with uniform distribution of lactic acid bacteria. The casein micelles were fused together, formed chains and clusters to entrap whey and lactic cultures. The use of starch as stabilizer results into rather stratified matrix without any discernible micro structural details suggesting interaction between starch and casein micelles (Roy, 1992).

**References**


Introduction

Milk and milk products constitute important nutritional components serve as the source of first class proteins especially for children and vegetarians. It supplies most essential elements like calcium and phosphorus along with numerous other essential major and minor substances. Among the Indian indigenous dairy products, khoa and khoa based milk sweets, paneer, Channa based sweets are provide a good means of conserving and preserving surplus milk solids (Karthikeyan and Pandiyan, 2013). The manufacture of these products is based on traditional method without any regard to the quality of raw material used and/ or the hygienic quality of the products. Under such conditions many microorganisms can find access to the milk products (Soomro et al., 2002). The unhygienic conditions at the production units lead to contamination of products with different types of microorganisms leading to a low shelf life of the finished products, most of the products are sold in the market without proper packaging and unduly exposing them to atmospheric contamination (Khan, 2006). Plethora of studies carried out in different part of India evidenced that pathogenic organism as Staphylococcus aureus, Bacillus cereus, often contaminate Khoa (Gill et al. 1994; Mandokhot and Garg, 1986). Probably the microbe’s access to Khoa is mainly by improper handling of workers and contaminated utensils used during processing (Bhatnagar et al. 2007). Contamination of Khoa and khoa based sweets, paneer, Channa based sweets by pathogenic bacteria could be an important factor of gastrointestinal infections including food poisoning and food borne illness.

The microbiological safety of food is of fundamental importance to all those companies and government organizations involved in the production, processing, distribution, retail and regulation of traditional milk products. Although quality, portion size, packaging format and other such issues are open to choice and commercial decisions, issues associated with the control of safety and pathogenic microorganisms are essentially “non-negotiable”.

There have also been significant developments in the approach to the microbiological safety control measures applied to food. Essentially, these measures were based on Good Manufacturing Practice (GMP) and Good Hygienic Practice (GHP) and on the implementation of a thorough Hazard Analysis Critical Control Point (HACCP) system. Although these tools are equally important today, a range of other objective control measures and risk assessment procedures are steadily being adapted to varying degrees by both government and industry. This chapter provides a more in-depth review of the tools available to support the application of MRA.

Microbiological Risk Assessment

Risk assessment for food safety sits within the framework of “risk analysis”, provided by the Codex Alimentarius Commission (Codex), which also includes “risk management” and “risk communication” as interdependent concepts. Risk assessment takes place within a risk management context, to aid decision-making on managing a microbiological hazard, and considers knowledge on the nature of the hazard and the likelihood of exposure to that hazard (CAC, 1999). In a more recent Codex guidance, the concept of a “risk profile” has been adopted under the Codex risk analysis
approach as part of the “preliminary risk management activities” and is “a description of the food safety problem and its context” (CAC, 2007). A risk profile may be considered a structured “narrative” type of evaluation, or a “preliminary risk assessment”. In addition to scientific information, other considerations such as public perceptions, trade impacts and management/intervention options may also be included in the document (Lammerding, 2007). Another relatively recent development has been the introduction of the concept of a “Food Safety Objective” (FSO) criterion to link food risk assessment to risk management (ICMSF, 2002; Stringer, 2004, 2005). The core elements of an MRA, i.e. hazard identification, hazard characterization, exposure assessment and risk characterization, are outlined in more detail below.

**Hazard identification**

Hazard identification is the first step in risk assessment. Hazard identification is defined in the Codex (CAC, 2004) as “The identification of biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods” (Lammerding et al., 2001). Hazards can be identified from publically available information such as published literature, epidemiological studies, foodborne disease reports, etc.

**Hazard characterization**

Hazard characterization is defined in the Codex “Procedural Manual, Fourteenth Edition” (CAC, 2004) as “The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food. For chemical agents a dose–response assessment should be performed. For biological or physical agents a dose–response assessment should be performed if the data are obtainable”. In MRA, this step provides a qualitative or quantitative description of the severity and duration of adverse effects that may result from the ingestion of a microorganism or its toxin in food. When establishing a dose–response relationship the different end points, such as infection or illness, should be taken into consideration (CAC, 1999).

Mathematical modeling of the dose-response relationship is recognized as a useful adjunct to the descriptive analysis of clinical or epidemiological information or data relating to foodborne illness. A microbiological dose-response model describes the probability of a specified response from exposure to a specific pathogen (or its toxins) in a specified population as a function of the ingested dose. The biological basis for microbiological dose–response models derives from major steps in the disease process: exposure, infection, illness and consequences (recovery, sequelae or death). The issue of response derives from the interactions between the pathogen, the host and the food matrix. Current thinking is that a single viable infectious pathogenic organism is able to induce infection (the “single-hit concept”) (FAO/WHO, 2003).

**Exposure assessment**

Exposure assessment is defined in the Codex “Procedural Manual, Fourteenth Edition” (CAC, 2004) as “The qualitative and/or quantitative evaluation of the likely intake of biological, chemical and physical agents via food as well as exposures from other sources if relevant”. Exposure assessment in MRA includes an assessment of the extent of actual or anticipated human exposure to microbiological pathogens or microbiological toxins, i.e. an estimate of the likelihood of their occurrence in foods at the time of consumption and their level, within various levels of uncertainty (CAC, 1999). Qualitatively, foods can be categorised according to the likelihood that the foodstuff will or will not be contaminated.
Risk characterization

Risk characterization is defined in the Codex “Procedural Manual, Fourteenth Edition” (CAC, 2004) as “The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment”. Risk characterization brings together all of the qualitative or quantitative information of the previous steps to provide a soundly based estimate of risk for a given population.

Microbiological risk profiling of traditional dairy products

The assessment of risks to public health and safety from microbiological hazards in milk and milk products has been undertaken in the form of a Microbiological risk profile. It provides a broad overview of risks associated with consumption of dairy products. The risk profile identifies key food safety hazards and assesses where in the primary production and processing supply chain these hazards might be introduced, increased, reduced or eliminated.

This risk profile identifies the microbiological public health and safety risks associated with dairy products. In compiling the risk profile, a wide range of scientific literature, data and information was reviewed and evaluated. When examining each dairy commodity category, only those potential pathogens relevant to the commodity being evaluated were assessed. The estimate of the severity of adverse health effects caused by a food-borne agent is based on the ranking scheme for food-borne pathogens and toxins described by the International Commission on Microbiological Specifications for Foods (ICMSF, 2002). The ICMSF ranking scheme categorizes hazards by the severity of the threat they pose to human health, taking into consideration the: likely duration of illness; likelihood of death; and potential for ongoing adverse health effects. The severity of adverse health effects caused by a hazard is ranked as moderate, serious or severe according to the following definitions:

<table>
<thead>
<tr>
<th>Severity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>Not usually life threatening; no sequelae; normally short duration; symptoms are self-limiting; can be severe discomfort</td>
</tr>
<tr>
<td>Serious</td>
<td>Incapacitating but not life threatening; sequelae infrequent; moderate duration</td>
</tr>
<tr>
<td>Severe</td>
<td>Life threatening, or substantial sequelae, or long duration</td>
</tr>
</tbody>
</table>

Under the ICMSF ranking, severe hazards are further divided into those applying to the general population and those applying to specific sub-populations, that is, susceptible individuals (for example, the very young and old, the immunocompromised, and pregnant women and their unborn children). This takes into account those situations where a hazard considered to be of moderate or serious to the general population may cause a severe illness in certain susceptible sub-populations. A brief summary of the micro-organisms, their severity of associated illness and the availability of epidemiological data is depicted in table -1

Microbial pathogens of major concern

Pathogens of major concern in khoa

Khoa, like other indigenous products such as chhana, kheer, dahi, etc., can serve as a favorable medium for the growth of a variety of microorganisms because of high moisture content and good nutritive value. The market khoa keeps well for 48 hours under usual Indian conditions of handling
and storage. However, storage beyond this period often results into deterioration due to microbial action. These microorganisms gain access into this product as contaminants from different sources. The rapid spoilage of khoa is attributed to contamination with moulds from external sources. A number of pathogens like *E. coli*, *S. typhi*, *S. dysenteriae* and *V. cholerae* are

Table - 1 Summary of micro-organisms considered in the risk profile

<table>
<thead>
<tr>
<th>Organism</th>
<th>Shed directly in milk#</th>
<th>Contaminant of raw milk##</th>
<th>Survives pasteurization</th>
<th>Severity of illness §</th>
<th>Dairy/ dairy products implicated in food-borne illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas spp.</td>
<td>×</td>
<td>✓</td>
<td>×</td>
<td>Serious</td>
<td>+</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>×</td>
<td>✓</td>
<td>✓</td>
<td>Moderate</td>
<td>++</td>
</tr>
<tr>
<td>Brucella spp.</td>
<td>✓</td>
<td>✓</td>
<td>×</td>
<td>Severe</td>
<td>+</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>×</td>
<td>✓</td>
<td>×</td>
<td>Serious</td>
<td>++</td>
</tr>
<tr>
<td>Clostridium</td>
<td>×</td>
<td>✓</td>
<td>✓</td>
<td>Severe</td>
<td>+</td>
</tr>
<tr>
<td>Clostridium</td>
<td>×</td>
<td>✓</td>
<td>✓</td>
<td>Moderate</td>
<td>+</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>✓</td>
<td>✓</td>
<td>×</td>
<td>Serious</td>
<td>+</td>
</tr>
<tr>
<td>Coxiella burnetii</td>
<td>✓</td>
<td>✓</td>
<td>×</td>
<td>Serious</td>
<td>+</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>×</td>
<td>✓</td>
<td>×</td>
<td>Severe</td>
<td>+</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>×</td>
<td>✓</td>
<td>×</td>
<td>Severe</td>
<td>++</td>
</tr>
<tr>
<td>Pathogenic <em>E. coli</em></td>
<td>×</td>
<td>✓</td>
<td>×</td>
<td>Severe</td>
<td>++</td>
</tr>
<tr>
<td>Listeria</td>
<td>✓</td>
<td>✓</td>
<td>×</td>
<td>Severe</td>
<td>++</td>
</tr>
<tr>
<td>Mycobacterium avium</td>
<td>×</td>
<td>✓</td>
<td>×</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Mycobacterium</td>
<td>✓</td>
<td>✓</td>
<td>×</td>
<td>Severe</td>
<td>+</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>×</td>
<td>✓</td>
<td>×</td>
<td>Serious</td>
<td>++</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>×</td>
<td>✓</td>
<td>×</td>
<td>Serious</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>✓</td>
<td>✓</td>
<td>×</td>
<td>Moderate</td>
<td>++</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>✓</td>
<td>✓</td>
<td>×</td>
<td>Serious</td>
<td>+</td>
</tr>
<tr>
<td>Yersinia</td>
<td>×</td>
<td>✓</td>
<td>×</td>
<td>Serious</td>
<td>+</td>
</tr>
</tbody>
</table>

# Transmission through udder; mastitis etc; ## via faeces, the environment etc; *Neurotoxin is heat labile; ** Enterotoxin is heat stable; ^ for vulnerable populations; § based on ICMSF (2002) severity ranking; + Reported, but rare; ++ More commonly associated with food-borne illness; − No data/unknown

able to survive for long periods during storage of khoa. Subsequently, a number of related studies have revealed the occurrence of staphylococci, especially those of coagulase positive types in khoa. The staphylococcus has been known to produce heat stable enterotoxin in this product which causes food poisoning. Since the product is manufactured by traditional method without any regard to quality of raw material used and hygienic storage, the shelf life of the product is adversely affected.
by the thermoduric organisms and organisms acquired during storage. High nutritional value and high water activity (aw = 0.96) of khoa is conducive to the growth of bacteria. A plethora of studies carried out in different part of India evidenced that pathogenic organism as B. cereus, S. aureus often contaminate khoa. Probably the microbe’s access to khoa is mainly by improper handling of workers and contaminated utensils used during processing. In a study total fifty samples of khoa were brought from different localities of Chambal region at random and processed. Bacterial colony counts were also performed. Staphylococcus species and Streptococcus species were the predominant isolates. Contamination of khoa by pathogenic bacteria could be an important factor of gastrointestinal infections including food poisoning and food borne illness. E.coli was isolated from milk products like mawa/ khoa, cream, dahi, cheese, butter and gulabjamun. In 2002, Soomro et al. observed that 12 (60%) of mawa/ khoa samples were contaminated with E. coli in contrast to 11 (55%) of dahi followed by 8 (40%) gulabjamun samples. Microbiological quality of market milk sweets in twin cities of Hyderabad and Secunderabad and observed 90% of peda, 75% of kalakand and 100% of rasagolla samples were contaminated with yeasts and moulds. Peda in general had more bacterial contamination than burfi contaminated with E. coli, P. aerogenes, S. flexneri, S. schottmuelleri and hemolytic streptococci. About 50 samples of peda randomly collected from various shops of Amravati city and analysed for bacteriological quality. Out of 92 strains of bacteria identified, the prominent were P. aerogenosa (23.91%), S. aureus (17.39%), S. typhi (16.30%), E. coli (14.13%), E. aerogenes (11.9%), Shigella flexneri (8.69%), Proteus vulgaris (7.6%), etc.

**Microbial pathogens of major concern in Paneer**

The possible sources of contamination might be air, water, utensils, cutting knife, muslin cloth as well as persons handling the product. Hence the number and types of microorganisms and their distribution in the product may vary depending on the location of the sweetmeat maker (halwai) shop, extent of exposure of the product to the atmosphere, temperature and period of storage, etc. Despite a higher final temperature (62°C) the duration of heating employed in the manufacture of Indian cheese (Paneer) was not sufficient to inactivate E. coli O157:H7. In a study 60 market dairy food samples, one sample each of raw milk, paneer and ice cream were found to be positive for E. coli O157:H7 with respective RT-PCR counts of 6.7, 6.2 and 5.9 log CFU respectively. Paneer is used in the preparation of certain curries and about 5% of the milk produced is converted to paneer. It may contain as high as 70% moisture which is conducive to microbial growth. Studies carried out on microbial quality of paneer have indicated that it is often contaminated with S. aureus and coliforms. The HACCP has been applied to identify the Critical Control Point for coliforms and Staphylococcus contamination.

**Microflora of major concern in Chhana**

Chhana is one of the two chief bases (the other being khoa) for preparing a variety of indigenous sweetmeats. Chhana is also called paneer in certain parts of the country. The PFA and ISI definitions of paneer also apply to chhana. Chhana samples showed an average bacterial count of 1.6 x10^4 per gram. However, during storage at 37°C, the count increased to 31x10^6 and 110 x 10^6 at the end of 24 and 48 hours, respectively. The spoilage of product was chiefly due to thermoduric bacteria. Among the bacterial types isolated from chhana micrococci predominated and constituted 45% of the total microflora followed by spore formers (34%).

**Attribution of food-borne illness to dairy products**

While there is enhanced quantitative data on the incidence of illness due to specific pathogens, there is often not the ability or capacity to identify or distinguish specific food vehicles. The causative agent of an illness is usually determined through epidemiological studies, but confirming the identity
of a key ingredient or the original source of product contamination, or critical factors contributing to their occurrence is problematic. This inability to attribute cases of food-borne illness to causal vehicles is a major issue internationally, and is especially difficult where illness is linked to foods with multiple ingredients. Problems arise because of difficulties with:

- Food recall biases when gathering food consumption histories;
- Long exposure windows with specific pathogens;
- Inability to obtain representative food samples for analysis; and
- A lack of precision in or suitable methods for, sample analysis.

**Risk Management Issues and Control Strategies for Dairy Products**

The critical factors having the most significant impact on the safety of processed dairy products are as follows:

- **The** quality of raw materials
- **Correct** formulation
- **Effective** processing
- **The** prevention of recontamination of product
- **Maintenance** of temperature control through the dairy supply chain.

While pathogenic microorganisms may contaminate raw milk supplies, pasteurization is a very effective Critical Control Point (CCP) in eliminating pathogens; good manufacturing practices must also be employed to ensure that post-pasteurization contamination does not occur. The effectiveness of pasteurization is dependent upon the microbiological status of the incoming raw milk. Control measures at the primary production level involve minimizing the likelihood of microbiological hazards contaminating the raw milk. This is achieved through the implementation of a food safety program incorporating good agricultural practices (GAP). These measures are effective in reducing the microbial load of milk being sent for processing.

However, should microbial contamination of raw milk occur, it is critical that milk is stored at a temperature that minimizes the opportunity for the bacteria to multiply. Temperature abuse of the milk may allow growth of pathogenic bacteria to the extent where the pasteurization process may not eliminate all pathogenic bacteria and/or toxins. The Afatoxins can be formed and ingested by dairy cattle during feeding, eventually contaminating the milk. Afatoxin contamination of milk is more common where intensive supplementary feeding of dairy herds is conducted.

**References**


Bacterial Spore Based Bioassay for Detection of Contaminants in Milk

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Introduction

Milk, being a major constituent of the human diet, its quality assurance is considered essential to the health and welfare of community. Now a day’s consumer demand for a product that has consistent quality, good taste and a longer shelf life and most importantly safe for consumption will ultimately benefit dairy producers by increasing consumption of this type of high quality products. Therefore the standards for a number of microbial and non-microbial contaminants have been specified for the first time as a legal requirement by Food Safety & Standards Authority of India (FSSAI) for milk and milk products in India. The testing of milk and milk products for presence of contaminating agents has become a mandatory practice for dairy industry before dispatching the products into the global market. Till date the conventional testing methods are considered as gold standards and are of first choice of the quality control laboratory of every dairy industry. But these conventional procedures are laborious to perform and more importantly requires 2-7 days on an average for complete testing of a milk product. But the industry cannot hold the product for so long time and hence by the time product is pushed into the market. The only thing that industry can do is banning the product and recalling the product. To overcome this unmanageable situation dairy industry is looking for alternatives to conventional methods which are rapid, cost effective, and easy to perform and significantly validated with approved standard methods. Our laboratory is playing a prominent role in this field by developing spore based detection system for monitoring microbial and non-microbial contaminants in milk and milk products and thus paving the way for ensuring fresh and healthy milk for consumption.

Spore: The basic structure

The members of genera Clostridium and Bacillus have the aptitude to form endospores during stress and starvation conditions. The dormant spore state of spores is very much stable allowing them to survive even for millions of years. The typical structure of spore is responsible for its survival ability even millions of years in dormant state (Desnous, Guillaume, & Clivio, 2009) as shown in Fig 1.

Figure 1: Spore Structure

The spore structure comprises of number of layers namely outermost cortex made up modified peptidoglycan. The next layer to cortex is multilayered protein shell known as coat divided into inner coat and an outer coat. The inner coat is casing the nucleic material in condensed state complexed
with small acid soluble proteins SASPs, which make up more than 20% of the spore protein composition (Peter Setlow, 2007).

**Spore Germination**

The irreversible process of spore germination is usually triggered by presence of signaling molecules in the environment marking the conversion of dormant spores into metabolically active vegetative cell. The signaling molecules which naturally induce germination are nutrients termed as germinants. The chemical nature of these germinants include are single amino acids, sugars or purine nucleosides, combinations of nutrients can also lead to spore germination as mixture asparagine, glucose, fructose and K+ (AGFK) triggers spore germination in B. subtilis (Paidhungat & Setlow, 2002). The germinants are species and strain specific for spores. Nutrient induced germination involve specific receptors (GRs; so named for the best studied GR in B. subtilis, GerA) localized in the inner membrane which are activated by germinants by allosteric interaction which are located in the inner-membrane of the spore (Hudson, Corfe, Kemp, Feavers, Coote, & Moir, 2001; Paidhungat, Ragkousi, & Setlow, 2001; Wolgamott & Durham, 1971). The binding of germinants to specific receptors initiates a series of events such as loss of refractivity well synchronized with the release of Ca2+DPA, a rapid efflux of monovalent cations (H+, Na+ and K+) and water to the core resulting in a partly dehydration (De Chen, Huang, & Li, 2006; Hashimoto, Frieben, & Conti, 1969). Afterward, there occurs removal of physical constraints i.e. spore layers by release of cortex lytic enzymes and allow core to expand (Popham, Helin, Costello, & Setlow, 1996)

**Exploiting Spore as Biosensing Element**

The presence or absence of microbial and non-microbial contaminants affects the spore germination phenomenon in two different ways. The presence of non-microbial contaminants i.e. antimicrobials such as antibiotics, aflatoxin and pesticides in milk impede the spore germination and restrict spore from releasing germination mediated enzymes and Ca-DPA even if germinant is present, this phenomenon is known as spore germination inhibition principle. While the microbial contaminants such as Enterococci and Listeria monocytogenes in milk act on specific complex sugars and convert them to simple sugars by their specific marker enzymes action. These simple sugars act as germinants and lead to spore germination as the presence of microbial contaminants in milk argument the spore germination and can be detected. This principle is known as germinant-germinogenic substrate principle.

**Advantages of using spore as biosensing elements**

The spore based biosensing systems are much superior in terms their activity and viability. Firstly spore based biosensing system has a long shelf life, as according to the studies of (Sangal, 2010) analytical performance of the spore-based sensing systems, was retained up to a period of 8 months when kept as dried spores at room temperature. Secondly, the spore germination process completes within minutes of sensing germinants in the environment so it can produce a real time response for detection of analyte. Thirdly, the spore production is a low priced process and its immobilization is an effortless process which curtails the cost of biorecognition molecule employed in a biosensor. Based on above characteristics spores were employed as vehicles to preserve, store and transport the whole-cell bacterial biosensing systems (Date, Pasini, & Daunert, 2007).

**Detection of aflatoxin M1 in milk**

For detection of aflatoxin M1 in milk a spore inhibition based –enzyme substrate assay (SIB-ESA) has been developed and patented (Patent Reg # 3064/ DEL/ 2010). The system comprises of spores of Bacillus spp. 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 germination mediated enzyme(s) are released by spores as milk act as germinant. The released enzymes act specifically on chromogenic/or fluorogenic substrate resulting in colored reaction/ or fluorescence as end product which is measured semi-quantitatively by either visually/ or using optical system at specific excitation/emission spectra (Fig.2). The milk containing analyte halts the spore germination phenomenon and no color development or fluorogenic reaction is detected. The developed system is capable of detecting the analyte at Codex recommended concentrations of aflatoxin M₁ (0.5 ppb) and works well with raw, pasteurized and dried milk products (Kumar et al. 2010; Singh et al. 2013).

![Fig. 2. Aflatoxin M₁ kit: Test Procedure](image)

Detection of β-lactam antibiotics in milk

A similar system for monitoring of β-lactam antibiotics in milk has been invented (Patent Reg No. 115/DEL/2009). 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 (Das et al. 2011). 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 (Gaare, Kumar, Raghu, Khan, & Singh, 2012).

![SAMPLE PREPERATION](image)

![TEST PROCEDURE](image)
Spore germination based assay for monitoring antibiotic residues

Assay involves the transformation of dormant spores of Bacillus stearothermophilus 953 into active vegetative cells. The inhibition of germination process specifically in presence of antibiotic residues was used as a novel approach for monitoring target contaminants in milk. The indicator organism i.e., B. stearothermophilus 953 was initially allowed to sporulated by seeding in sporulation medium and incubating at 55°C for 18 ± 2 h. The spores exhibited a typical chain behavior as revealed through phase contrast microscopy. The minimal medium inoculated with activated spores was incubated at 64°C for 2–3 h for germination and outgrowth in presence of specific germinant mixture containing dextrose, whey powder and skimmed milk powder added in specific ratio along with reconstituted milk as negative control and test milk samples. (Fig. 3).

Novel Features of MDR test

- The Cost effective (Rs 25 per test )
- Semi-quantitative detection at Codex MRL
- Validated with AOAC approved Charm 6602 Assay (Fig. 2)
- Minimal false positive /negative results
- Consistency in color development within 3.0 h
- No interference of inhibitors other than antibiotic residues
- Stability of test kits up to 12 months under refrigeration storage
- Wide scope of application to raw, pasteurized and dried milks
- Test kit can perform at dairy farm, milk collection center, dairy reception dock and R&D institutions

Detection of Microbial Contaminants in Milk

Each year various food borne illness outbreaks and food recalls are reported because of actual or potential bacterial contamination. In view of this Food Safety Standards Authority of India (FSSAI) has made it mandatory for the food and drugs department of every state to test for harmful bacteria like E. coli, S.aureus and Listeria monocytogenes before pushing it into the market. The producers will have to declare on their packets that the milk is certified free of the any microbial contamination.

Spore based biosensor for detection of L.monocytogenes

The spore based detection system consist of two stage assay firstly the primary enrichment of milk sample in a developed selective medium, Listeria selective enrichment medium (LSEM) which allow selectively the growth of L. monocytogenes while inhibit all other potential contaminants (Mandeep et al. 2013; Mandeep, 2013). During this step there will be a change in color of the medium i.e. from yellow to black/ or blue, indicating the presence of Listeria spp. based in marker enzyme activity and is used as an indication for detection of Listeria spp(Fig. 4 ).This enrichment phase is termed as Stage -1.
After enrichment of cells, cells are pellet out and washed with buffer thrice to remove medium components. The pure cells obtained are used to perform spore based assay which consist of incubating cells with *Bacillus* spores and specific combination of complex sugars acting as germinogenic substrates. These sugars combination is specific for specific marker enzymes *L. monocytogenes*. The marker enzymatic activity of *L. monocytogenes* acts on complex sugars to convert it into simple sugars which can act as germinants. The germinants prop up germination activity in *Bacillus* spores which is detected by cleavage of fluorogenic substrate specific for germination mediated enzymes i.e. diacetate fluorescein (DAF).

**Spore based biosensor for detection of Enterococci:** Spore based detection system for rapid detection of Enterococci is based on targeting β-D-glucosidase as marker enzyme and its specific action on marker-enzyme substrate i.e. esculin resulting in germinant stimulus for spores produced by specific strain of *B. megaterium*. The developed spore based bioassay consists of target bacteria, microbial spores suspended in buffer, marker-enzyme substrate and a fluorogenic substrate. The detection principle is based on quantification of fluorescent signal produced as result of DAF hydrolysis by germination mediated marker enzyme released from bacterial spores germination triggered by β-D-glucosidase activity on non-specific germinant substrate. The spore based bio-assay developed has sensitivity of 5.5 ± 0.4 log cells after pre-enrichment of milk sample in specific enrichment broth i.e. sodium Azide & esculin based medium (SAEBM) within real time of 8 ± 2 hrs (Fig. 5) (Kaur, 2011; Deshmukh, 2013).

**Conclusion**

The living phase of bacterial spores revolves around two phases i.e. dormant state and metabolically active vegetative state. This conversion from one phase to another phase is completed only if spore senses favorable conditions in the environment and presence or absence of microbial or non-microbial contaminants directly or indirectly affect this conversion. So this phenomenon can be targeted to sense the presence of contaminants in milk and hence develop spore based biosensor systems. A number of spore based sensing system have been developed to detect aflatoxin, antibiotics and microbial pathogens in milk. These biosensing systems are superior over existing methods in terms of better sensitivity, low cost and help in rapid analysis of milk and milk products. The spore based biosensor is a novel strategy being exploited to ensure safe and healthy milk to each consumer.
5a. Novel Microtechniques for detection of Enterococci

1. Add 950 µl of EBSAM to a clean test tube
2. Add 50 µl of milk sample to 950 µl of EBSAM
3. Incubate the tubes at 37 °C
   Observe color change after every 1 hour interval

5b. Selectivity of EBSAM

- Black color indicates presence of Enterococci
- No color change indicates absence of Enterococci

References


Enhancement of Shelf Life of Indigenous Dairy Products Using Phytochemicals

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Introduction

Milk is an extremely complex biological fluid that contains water, lactose, fat, proteins and minerals, secreted by mammary gland of female mammals. These chemical nutrients present in fluid exist in three physical phases: a dilute emulsion, colloidal dispersion and a solution. The chemical makeup of milk and its physicochemical behaviour provide scientific basis for process of milk and manufacture of products. Milk is sole source of energy for the very young ones as it provides nourishment and immunological protection. This is a highly perishable commodity in its native state because of its susceptibility to rapid spoilage by the action of naturally occurring enzymes and contaminants. Milk and milk products are prone to contamination by spoilage and pathogens at various stages of production, processing, post processing transportation & handling. Although, we are the highest milk producer but lag behind far below international standards in microbial quality that result about 5% loss due to souring of milk. Contamination of raw milk with pathogenic and spoilage microflora make it unfit for consumption and processing, causing huge economic loss to producers products. In India, milk is obtained from buffalo and cow (desi, cross breed and exotic. Depending on its characteristics, each type of milk is eminently suitable for certain types of region specific indigenous traditional milk. Indian traditional dairy products can be grouped as heat desiccated (khoa, gulabjamun, burfi, peda, kalakand) heat & acid coagulated (paneer, chhana, rassogolla, sandesh, cham cham, rasmalai, pantoha, raj bhog, chhana murki), fermented (dahi, chakka, shrikhand, misti dahi, lassi) and fat rich (malai or cream, makkan, ghee). Keeping quality of ghee from cotton seed fed animal is better due to the presence of gossypol – a phenolic substance present in cotton seed which act as antioxidant.

There is a growing awareness that microbial contaminants in dairy foods may play an etiological role in various human diseases and cause huge economic loss due to spoilage. Thus, food safety and security is great health and economic concern, which cannot be taken lightly. It has been estimated that as many as 30% of people in industrialized countries suffer from food borne diseases each year (WHO, 2000). Microorganisms play a major role in contamination of stored food deteriorating quantitatively and qualitatively. Yeast and molds destroy dairy foods during storage, rendering them unfit for human consumption by affecting their nutritive value and sometimes produce mycotoxins. These are secondary metabolites of filamentous fungi especially Aspergillus flavus and A. parasiticus. Aflatoxins are stable under normal food processing conditions and can therefore be present not only in food and feed, but also in processed products and threaten both human and animal health as they are known to be carcinogens. Among these aflatoxin M1 (AFM1) is the most significant in terms of human health risk. Physical, chemical and biological methods have been tried in order to prevent growth of contaminants to eliminate or reduce risk of food poisoning and deterioration. Extracts and powders of various spices, herbs and essential oils have been reported to have antimicrobial activity against spoilage and pathogenic microflora and many essential oils have also been reported as effective inhibitors. Plants essential oils are potentially valuable sources of antimicrobial compounds. Several studies have been published on the antimicrobial activities of plant compounds against food-borne pathogens and spoilage microorganisms. The main components of
essential oils are mono and sesquiterpenes. Owing to these properties, spices and herbs have been added to food since ancient time, not only as flavoring agents but also as preservatives.

**Phytochemicals**

These are large group of plant bioactive secondary metabolites used to protect the plants from various environmental challenges including microbial invasion. These also exert various health benefits in humans. The term is generally referred to those chemicals which have biological significance e.g. antioxidants and antimicrobials but are not established as essential nutrients. These are found in plant-based foods such as fruits, vegetables, beans, and grains. There is some evidence that a diet rich in fruits, vegetables, and whole grains reduces the risk of certain types of cancer and other diseases. Researchers are looking for specific compounds in these foods that may account for these healthful effects in humans. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. There are more than thousand known phytochemicals.

**Health benefits of phytochemicals**

The phytochemicals exert their health benefit by one of the following possible actions:

- **Antioxidant:** Most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer e.g. allyl sulphides (onions, leeks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphones (tea, grapes).

- **Hormonal action:** Isoflavones, found in soy, imitate human estrogens and help to reduce menopausal symptoms and osteoporosis.

- **Stimulation of enzymes:** Indoles, which are found in cabbages, stimulate enzymes that make the estrogen less effective and could reduce the risk for breast cancer. Other phytochemicals, which interfere with enzymes, are protease inhibitors (soy and beans), terpenes (citrus fruits and cherries).

- **Interference with DNA replication:** Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin, found in hot peppers, protects DNA from carcinogens.

- **Anti-bacterial effect:** These act as selective inhibitors of deleterious intestinal bacteria. The phytochemical allicin from garlic has anti-bacterial properties.

- **Physical action:** Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. Proanthocyanidins of cranberry possess anti-adhesion properties. Consumption of cranberries will reduce the risk of urinary tract infections and will improve dental health.

- **Selective growth factors for beneficial gastrointestinal bacteria:** fermentation substrates for beneficial oral, gastric or intestinal bacteria e.g. terpenoids, phenolics, alkaloids and fiber.

**Types of phytochemicals**

Based on their chemical structure they can be broken into the following groups depicted in picture.
Requirements of food antimicrobials and factors affecting their efficacy

It is estimated that 250,000-500,000 species of plants exist on earth and only 1/10th of these have been explored for thousands of bioactive antimicrobial or medicinal compounds. As healthcare trends move toward disease prevention there will be higher market for functional foods containing natural bioactive antimicrobials, as consumers are choosing foods over pharmaceuticals for their well being. Essentials oils (EOs) are secondary metabolites obtained from various plant parts and have been used for centuries in complementary or alternative medicine for treatment of various ailments, perfumery and cosmetics. >3000 different EOs are known of which ~300 commercially used in flavour and fragrances market as these have been accorded GRAS status. Although, food industry primarily uses EOs as flavourings, however recently, these have been explored for food preservation and food safety due to their broad-range antimicrobial activities. Dela Croix in 1881 was the first to evaluate antimicrobial activity of plant EOs. The antimicrobial nature of phyto-chemical is determined by its chemical properties, such as pKa value, hydrophobi-city/ lipophilicity ratios, solubility, and volatility. The pH and polarity are the most prominent factors that influence the effectiveness of a food antimicrobial. Further, hydrophobic properties of some antimicrobial substances can make their dissolution difficult in water limiting their use in foods.

The application of bioactive components as food preservatives requires detailed knowledge about their properties, i.e., minimum inhibitory concentration (MIC), range of target organisms, mode of action, and effect of food matrix components on their antimicrobial activity. Generally, higher concentrations of natural antimicrobials are required to achieve the same effect in food as compared to in-vitro assays. Various intrinsic (pH, salt, antioxidants and other additives) and extrinsic properties (temp, vacuum and modified atmosphere packaging, characteristic of microorganisms) of food play a vital role in antimicrobial efficacy of bioactive components in food matrix as it affects the growth and behaviour of food borne pathogens and spoilage microbes. Utilization of bioactive components in single or in combination can be a novel alternate approach to achieve food safety and avoid spoilage as well as to minimise adverse effect on sensorial attributes of food. Cinnamon EO was the most effective under refrigerated temp, inhibiting the growth of *B. cereus* for at least 60 days in a model.
refrigerated minimally processed food product made with carrots and fractional heating. Lettuce leaf, beef and milk model media were used to study the efficacy of EOs against *Listeria* and spoilage bacteria, which was compared to the laboratory control media i.e. Tryptic Soy Broth (TSB). The efficacy of essential oils in the lettuce model media was 10 fold higher than in TSB, possibly due to the low fat content of vegetables. The EOs were less effective in beef extract than in TSB, probably high protein concentrations in beef extract promoted the growth of *Listeria* species. Oregano and thyme were the most effective for inhibition of *Listeria* and spoilage organisms for the above food model media tested.

**Natural antimicrobials for food uses**

As far as the use of natural antimicrobials in foods is concerned, the lack of reproducibility of their activity is one of the major obstacles, despite the great diversity of compounds they contain. Qualitative and quantitative variations in the content of bioactive phytochemicals in plant extracts result in their variable effectiveness. Further, the extrapolation of results obtained from *in-vitro* experiments with laboratory media to food products is not straightforward as foods are complex, multicomponent systems consisting of different interconnecting microenvironments. Though there is vast potential for natural antimicrobial agents in food preservation, most of the literature presents inactivation data from model foods or laboratory media. The level of natural preservatives required for sufficient efficacy may be considerably higher in food products in comparison with laboratory media, which may negatively impact the organoleptic properties of food.

**Essential oils & extracts**

Different studies have demonstrated the effectiveness of EOs and their active compounds to control or inhibit the growth of pathogenic and spoilage microorganisms and reported its dependence on pH, chemical structure and concentration used of bioactive compound, besides the number and type of microorganisms. The bacterial susceptibility to EOs increases with a reduction in pH of the food, since at low pH the hydrophobicity of the oil increases, enabling it to more easily dissolve in the lipids of cell membrane of the target bacteria (Burt, 2004). Partition coefficients of the EOs might also have an effect on activity by influencing its diffusion rate through the cell membrane, as higher partition coefficient of citral as compared to cinnamaldehyde and eugenol resulted in the faster reduction of *E. coli* O157:H7 (Raybaudi-Massilia et al., 2009). Storage temperature also influences the antimicrobial effectiveness of EOs, as the bactericidal activity of different EOs or their active components against *E. coli* O157:H7 and *Salmonella* in apple juice was higher at 37 °C than at 4 and 21°C (Friedman et al., 2004). Owen and Palombo (2007) investigated the ability of *Eremophila duttonii* and *E. alternifolia* to control the growth of *L. monocytogenes* in full cream milk, skim milk, diluted homogenates of salami, pate and brie cheese, and reported that both the extracts inhibited the growth of *L. monocytogenes* in salami at 37°C, only *E. Duttonii* extract was effective in pate at 4°C storage, and growth of *L. monocytogenes* was not affected by both the extracts in other products. In another study on yoghurt, addition of anise EOs and oleoresin at 1.0 g/L conc. is effective in controlling the growth of spoilage microbes without any adverse effect on physicochemical attribute and viability of LAB of yogurt microorganisms. Further, anise is advantageous because of its antioxidant and antimicrobial activity as well as nontoxic that can reduce oxidative degradation of fatty substances as well as inhibit growth of spoilage microflora (Singh et al., 2011).
Table 1: Natural active compounds applied to dairy products.

<table>
<thead>
<tr>
<th>Products and storage conditions</th>
<th>Natural compounds</th>
<th>Main results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>West African soft cheese</td>
<td>Treatment with eucalyptus oil and lemongrass oil</td>
<td>The treatment of eucalyptus oil 75% plus 25% lemon grass exerted a positive impact on the nutritional, sensory, and microbial values of West African soft cheese</td>
<td>Belewu et al., 2012</td>
</tr>
<tr>
<td>Ricotta cheese stored under modified atmosphere at 4°C</td>
<td>Coating with a chitosan/whey protein edible film</td>
<td>The viable numbers of lactic acid bacteria and mesophilic and psychrotrophic microorganisms were significantly lower in the chitosan/whey protein coated cheese, compared to the control</td>
<td>Di Pierro et al., 2011</td>
</tr>
<tr>
<td>Traditional Minas Serro cheese</td>
<td>Nisin</td>
<td>Nisin was effective in reducing S. aureus count in Serro cheese. A reduction of 1.2 and 2.0 log cycles in S. aureus count was observed from the 7th day of ripening for cheese containing 100 IU/mL and 500 IU/mL of nisin, respectively, compared with control sample</td>
<td>Pinto et al., 2011</td>
</tr>
<tr>
<td>Fresh cheese Tosèla</td>
<td>Antimicrobial compounds produced by six strains of non-starter lactic acid bacteria. In particular, Lb. paracasei NdP78 was also found to produce bacteriocin</td>
<td>Cheese showed higher conc of lactobacilli (7.90 log CFU/g) and streptococci (6.10 log CFU/g), lower development of coliforms and staphylococci than control cheese</td>
<td>Settanni et al., 2011</td>
</tr>
<tr>
<td>Caprese salad packaged under MAP(65%N2, 30% CO2, and 5%O2)</td>
<td>Dipping with thymol (400 ppm)</td>
<td>The combined use of thymol and MAP decreased the coliform populations from 5.65 to 4.23 log CFU/g and extend ed microbiological shelf-life from 3.77 to 12 days. It also decreased the conc of Pseudomonadaceae</td>
<td>Bevilacqua et al., 2007</td>
</tr>
<tr>
<td>Gorgonzola cheese</td>
<td>Natamycin-incorporated film in the production process of cheese</td>
<td>Films with 2 and 4% natamycin presented satisfactory results for P. roqueforti inhibition</td>
<td>de Oliveira et al., 2007</td>
</tr>
</tbody>
</table>

(Source: Lucera et al., 2012)
Essential oil (EO) constituent classes

EOs constituent is a diverse family of low mol. weight organic compounds with large differences in antimicrobial activity. Identification of bioactive component is cumbersome as these are complex mixtures of upto 45 different constituents as well as their composition may vary depending on the season of harvest, and methods of extraction (Espina et al., 2011; Paibon et al., 2011). The bioactive compounds are broadly divided into four groups according to their chemical structure: terpenes, terpenoids, phenylpropenoids, and “others.”

**Terpenes**

These are hydrocarbons produced from combination of several isoprene units (C$_5$H$_8$) and synthesized in the cytoplasm of plant cells. The main terpenes are monoterpenes (C$_{10}$H$_{16}$) and sesquiterpene (C$_{15}$H$_{24}$), but longer chains such as diterpenes (C$_{20}$H$_{32}$), triterpenes (C$_{30}$H$_{40}$). Examples include p-cymene, limonene, terpinene, sabinene, and pinene. In vitro tests indicate that terpenes are inefficient antimicrobials when applied as single compounds.

**Terpenoids**

Terpenoids are terpenes that undergone biochemical modifications via enzymes that add oxygen molecules and move or remove methyl groups. These can be subdivided into alcohols, esters, aldehydes, ketones, ethers, phenols, and epoxides (e.g. thymol, carvacrol, linalool, linalyl acetate, citronellal, piperitone, menthol, and geraniol). Antimicrobial activity of most terpenoids is linked to their functional groups. The hydroxyl group of phenolic terpenoids and presence of delocalized electrons are important for antimicrobial activity. Exchange of hydroxyl group of carvacrol with methylether affects its hydrophobicity and antimicrobial activity.

**Mode of action**

The mechanism of action of bioactive components is not fully understood. The Gram -ve are generally less susceptible than Gram +ve bacteria; as outer membrane of former contain hydrophilic lipo-polysaccharides (LPS), which create a barrier toward macromolecules and hydrophobic compounds providing them higher tolerance toward hydrophobic antimicrobials present in EOs. Since, most EOs constituents have several targets, thus it is difficult to predict variations in susceptibility of microbial strains. Terpenoids and phenolics results membrane disruption whereas phenols and flavonoids causes metal chelation. Coumarin and alkaloids affect genetic material that inhibits the growth of pathogens and spoilage microorganisms. Degradation of cell wall, damage to cytoplasmic membrane and membrane proteins, leakage of intracellular contents, coagulation of cytoplasm and depletion of proton motive force can cause cell death.

Thymol, eugenol, and carvacrol cause disruption of the cellular membrane, inhibition of ATPase activity, and release of intracellular ATP and other constituents of target microbes. Cinnamaldehyde produces a decrease in the intracellular ATP by ATPase activity. It also increases membrane permeability, leakage of cytoplasm and interact with enzymes located on cell membrane. These changes may cause its disruption to proton motive force by leakage of small ions or it can inhibit the enzymes necessary for amino acid biosynthesis. Carvacrol and thymol appear to make the cell membrane permeable by dissolving into the phospholipid bilayer and they get aligned between the fatty acid chains. This distortion causes expansion and destabilization of the membrane increasing its fluidity, which in turn increases passive permeability. Thymol binds to the membrane proteins hydrophobically and changes the permeability characteristics of membrane. Phenolics probably exert their toxic effects at membrane as high correlation exist between toxicity and hydro-phobicity. Phenol changes membrane functioning and influences protein-to-lipid ratios in the membrane and induces
efflux of potassium ions. The catechins have shown to disrupt membrane integrity, as they cause leakage from liposomes. These interactions in outer polar zone of lipid bilayers in liposomes cause membrane disruption. Vanillin showed antimicrobial effect by affecting membrane functions and through the inhibition of respiration in several bacteria. Terpenes accumulate in the membrane and cause a loss of membrane integrity and dissipation of the proton motive force as well as disrupt the lipid structures. Investigations on cell and vesicle systems confirm that p-cymene has no effect on the membrane permeability, but cause a decrease in the enthalpy and melting temperature of membranes, supporting the hypothesis that p-cymene act as a substitutional impurity in the membrane.

Both essential oils and their mixture showed broad antifungal spectrum against Aspergillus flavus, A. niger A. Fumigates A. terreus A. glaucus A. nidulan A. parasiticus A. Versicolor, Penicillium expansum P.citrinin Alternaria, Rhizopus oryzae R. stolonifer Fusarium oxysporum, important food contaminating fungi. The essential oils of G. glabra and M. chamomilla completely inhibited aflatoxin B1 (AFB1) production at 800 ppm. Both oils exhibited antioxidant activity as DPPH free radical scavenger in dose dependent manner. Percentage of radical scavenging activity of G. glabra and M. chamomilla oils at 400 μg/ml were calculated to be 85.2 and 91.7%, respectively as compared to standard (BHT) with 75.6% activity at the same concentration. The anticancer properties of essential oils against cells (MCF-7) were evaluated. In anticancer activity exposure of essential oils caused a significant decrease in cell viability in MCF-7 cell line (breast carcinoma). Exposure of MCF-7 cells with G. glabra essential oils resulted in dose dependent increase in cell growth inhibition (CGI) varying from 3 to 77% at concentration ranging from 10 to 640 μg/ml. Similarly, 7 to 89% CGI was obtained when M. chamomilla essential oils was used. The present study demonstrated that essential oils of G. glabra and M. chamomilla have potent antifungal, antioxidant, and anticancer with the presence of effective phytochemicals.

Stability of bioactive during food processing

Health promotion through diet is gaining importance at a very fast pace, therefore, understanding of processing effects on bioactive components is critical as they not only preserve the foods, but also have beneficial effect on human health. Heat processing i.e. sterilization, pasteurization, and dehydration may result loss while in some cases, induces the formation of the novel compounds, which either maintain or even increase the potential of various bioactive ingredients. Thermal processing caused marked losses in total anthocyanins in black raspberries and blueberries. The thermal stability of phytochemicals added to food depends on the matrix in which they are found and added. The presence of other polyphenolics and antioxidants in the matrix may help to stabilize these compounds. Significant changes in individual isoflavone levels were observed during storage of UHT processed chocolate flavoured high protein beverage containing soy proteins isolates depending on storage temperatures (4, 23 and 38 °C). Microcapsule curcumin was found to have similar antibacterial and antifungal activities as curcumin after microencapsulation. Similarly, modifications of curcumin by glucoside synthesis (Parvathy et al., 2009) or amino acid conjugation retained its antibacterial, antioxidant and antimutagenic activities, indicating that curcumin is stable to chemical modifications.

Toxicity evaluation

Majority of bioactive components of plant origin that find application in foods, have been consumed for thousands of years, however, typical toxicological information such as acceptable daily intake (ADI) or no observed adverse effect level (NOEL) are not available. Although International guidelines exist for the safety evaluation of food additives, however, due to problems in standardization of these components owing to their batch wise compositional variability, it is difficult
to assign ADI or NOEL. The marker compounds in EOs are affected by variety of plant, geographical origin, plant part used, age and growth condition of plants, method of extraction or drying, preparation, packaging and storage. According to Dietary Supplement Health and Education Act (DSHEA), 1994, botanicals are exempted from food additive category, and GRAS submission of safety evidence is not required as long as that ingredient was in market before October 1994.

The International Life Sciences Institute-Europe has developed a comprehensive document on the use of plant materials in food products (Schilter et al., 2003), which stresses that the ingredient for use in food products must be well identified and characterized. The starting material must be accurately identified in order to ensure that the plant materials for food use are consistent with respect to quality and quantity of active ingredient. Risk assessment of natural products may require adequate specification of identity and composition as it may be the whole plant, extracts thereof or purified components, and the variability among plant source and the process used to obtain the constituents will be a limiting factor in adopting a generic approach to their risk assessment. A decision tree has been suggested as an aid to the safety evaluation process for plant material intended for food use, and general framework for safety assessment of botanicals has been described (Speijers et al., 2010; van den Berg et al., 2011).

**Interaction of bioactive with food components and its effects on antimicrobial activity**

Most of investigations on antimicrobial properties of plant EOs have been focused at the efficacy against planktonic cultures of food related bacteria. However, evaluation for antimicrobial activity against native microflora of foods at different storage times, changes during processing and packaging is better criteria e.g. lightly preserved fish products where certain ingredients such as salt or sugar are added and mildly processed using cold smoke. This type of processing lowers the water activity thereby inhibiting the growth of spoilage organisms and enhancing the growth of lactic acid bacteria (LAB). Vacuum packing of meat inhibits the aerobic *Pseudomonas* sp. causing a change in microbiota to LAB and *Enterobacteriaceae*.

**Synergies within bioactive components**

The main difficulty in utilization of plant EOs in dairy foods is their higher concentration requirement that has adverse effect on sensorial quality due to strong aroma. Therefore, optimisation of their levels in foods is utmost priority. Bioactive components in combination exert better antimicrobial effect in foods as compared to individual major constituents, indicative of minor components synergy. Synergism between carvacrol and p-cymene in brain heart infusion broth suggests that a combination of weaker activity components can achieve a synergistic effect. An additive effect was observed when the combined effect is equal to the sum of the individual effects. Antagonistic effect is observed when the effect of one or both compounds is less when they are applied together than when individually applied. Synergism is observed when the effect of the combined substances is greater than the sum of the individual effects. Binary mixtures of carvacrol and thymol had synergistic activity against *L. innocua* in comparison to antimicrobial efficacy of the individual components. Green tea extracts alone (20 or 40 mg/ml) or in combination with tartaric acid (37.5 mM) reduced *Salmonella, Listeria* and *E. coli* by 3.5 log CFU/ml in broth culture. The antimicrobial activity of grape seed extract when combined with bacteriocins like nisin has demonstrated more effectiveness than when used alone against *L. monocytogenes*. This may be due to the synergistic mechanism of action of nisin and polyphenols present in grape seed extract.

**Delivery systems**

Many methods are available to incorporate plant bioactive into foods. The simplest method is direct addition, however, in foods where surface sanitisation is targeted food product can be dipped in
bioactive component(s) or applied as a spray. These simple delivery modes have become more sophisticated with the advancement in packaging, encapsulation and nanotechnologies. Some of the modes of application available for plant antimicrobials include:

**Bioactive packaging**

Incorporation of antimicrobial components in films rather than direct addition with food provides better functional effect at the food surface, where most of the microbial growth is localized. Antimicrobial packaging would include systems such as addition of sachet into package, dispersion or coating of bioactive in or on the surface or as part of edible packaging material. Incorporation of garlic oil at 100 μl/g in chitosan and forming a film was found to exhibit antimicrobial activity against *S. aureus, L. monocytogenes* and *B. cereus*. Kakadu plum (*Terminalia ferdinandiana*) is one of the Australian native fruits identified for its antimicrobial properties, having gallic acid as one of the components with antimicrobial efficacy. Films made from 3% kakadu plum powder were found to have antimicrobial activity against *S. aureus*, methicillin-resistant *S. aureus* (*MRSA*), *L. monocytogenes*, *B. cereus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and *Acinetobacter baumannii*.

**Encapsulation**

Application of phytochemicals as preservative in food depends on maintaining the stability and bioactivity of the plant antimicrobial. The adverse effect of strong aroma and taste of bioactive antimicrobials can be overcome by encapsulation instead of direct addition as free component to the food. The encapsulation technologies include spray drying, coacervation, liposome entrapment, inclusion complexation, co-crystallization, nano-encapsulation, freeze drying, yeast encapsulation and emulsions etc. Nano-encapsulation of bioactives represent an efficient approach to enhance physical stability, protect them from interactions with food ingredients and, improve their bioactivity due to sub cellular size. A mixture of terpenes and D-limonene was encapsulated into nano-emulsions based on food-grade ingredients, prepared by high pressure homogenization. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the nano-encapsulated terpenes against *E. coli*, *L. delbrueckii* and *S. cerevisiae* were lower or equal to the values of the un-encapsulated mixture. Microencapsulation of Mexican oregano EOs by spray drying preserved antimicrobial efficacy against *P. aeruginosa, S. aureus* and *E.coli*, improved its solubility in water. Linalool and methylchavicol from basil when incorporated in Low density polyethylene significantly reduced *E. coli* and *L. innocua* in Cheddar cheese.

**Challenges of using plant bioactive in foods**

Plant bioactive components are essential to inhibit natural micro-flora of foods. The challenges of using plant antimicrobials are:

- Some plant bioactive have adverse flavours effect associated with, therefore it is essential to match the food with bioactive flavour or understand the synergies to decide on the concentration to be used.
- Type of microorganisms present in the food that can cause spoilage and disease is critical to understand the antimicrobial effect of plant components as it is not the same for all microorganisms.
- Incorporation of plant antimicrobials in food can promote growth and virulence of certain pathogens due to changes in microbial ecology. It is critical to understand their effect on behaviour of these microorganisms in complex food systems.
- The growing environment of the source plants influences the levels of antimicrobial compounds in them. In addition, the period of harvest, storage and extraction procedures used have an effect on
the levels of active components responsible for antimicrobial activity and this would be a challenge in using it as a functional food ingredient.

Conclusions

Microbial food safety and prevention of its spoilage is still a major concern to health conscious consumers, regulatory agencies and food industries over the globe. Many food preservation strategies have been used traditionally for the control of pathogens and spoilage microflora but contamination of food and spoilage microorganisms is a continuous problem yet to be controlled adequately. The literature demonstrates that different plant antimicrobials effectively reduce or inhibit pathogenic and spoilage microbes, and thus have a potential to become a good alternative to synthetic preservatives. Further, the use of natural antimicrobials in combination with another or with other technologies in a multi-hurdle preservation system can enhance the performance of natural bio-actives. Natural antimicrobials offer unique advantages for traditional dairy foods w.r.t. improvement their shelf life and safety of foods. Their incorporation may allow developments of novel food products with enhanced food safety, shelf life and nutritional security. The applications of natural antimicrobial agents are likely to grow steadily in the future because of consumer demand for minimal processing and food containing naturally derived preservatives is on rise. Further, it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. The impact of product formulation and storage parameters on the efficacy of natural antimicrobials as well as safety and toxicology evaluation of these natural antimicrobials require an in-depth study.

References


Developments in Starter Culture Technology for Fermented Milk Products

Surajit Mandal
Scientist, Dairy Microbiology Division, NDRI, Karnal

Introduction

A starter culture can be defined as a microbial preparation of large numbers of cells of at least one microorganism to be added to a raw material to produce a fermented food by accelerating and steering its fermentation process. The group of lactic acid bacteria (LAB) occupies a central role in these processes, and has a long and safe history of application and consumption in the production of fermented foods and beverages. The production of lactic acid, by fermenting lactose is the major role of dairy starters. The acid is responsible for development of characteristic body and texture of the fermented milk products, contributes to the overall flavour of the products, and enhances preservation. Diacetyl, acetaldehyde, acetic acid, also produced by the lactic starter cultures, contribute to flavor and aroma of the final product. Carbon-di-oxide produced by some hetero-fermentative lactic acid bacteria involves in very characteristics texturization in some fermented dairy products, viz. “eye” formation in cheeses. Development of flavor and changes in texture during ripening of cheeses is associated with enzymes originating from bacterial and fungal cultures, depending on the cheese variety. Dairy starters are also having some direct or indirect functional health promoting attributes, such as live probiotics, prebiotic exopolysaccharides and oligosaccharides, bioactive peptides and lipids, etc. Most of the cultured dairy products are produced using commercial starter cultures that have been selected for a variety of desirable properties in addition to rapid acid production. These may include flavor production, lack of associated off flavors, bacteriophage tolerance, ability to produce flavor during cheese ripening, salt tolerance, exo-polysaccharide production, bacteriocin production, temperature sensitivity, etc.

Types of starter cultures

In the dairy industry, single or multiple strains of cultures of one or more microorganisms are used as starter cultures. These are belongs to genus Lactococcus (Lactococcus lactis subsp. cremoris, L. lactis subsp. lactis, L. lactis biovar diacetylactis), Lactobacillus (L. delbrueckii subsp. lactis, Lactobacillus acidophilus, Lactobacillus casei), Streptococcus (S. thermophilus), Leuconostoc, Pediococcus etc. There are two main types of lactic starters:

1) Mesophilic lactic starters (optimum growth temperature: 30°C)
2) Thermophilic lactic starters (optimum growth temperature: 42°C)

Mesophilic cultures usually contain L. cremoris and L. lactis as acid producers and L. diacetylactis and Leuconostocs as aroma and CO₂ producers. Thermophilic starters include strains of S. thermophilus, and, depending on the product, Lactobacillus bulgaricus, L. helveticus, or L. lactis. Often, some fermented milks made with thermophilic starters also contain Lactobacillus acidophilus, L. bulgaricus and bifidobacteria for their healthful and therapeutic properties. Table 1 lists the common starter cultures and their applications in cheese and fermented dairy products.

The lactic starter cultures are also subdivided into two groups:

1) Defined cultures; 2) Mixed cultures.
Defined cultures constitute starters in which the number of strains is known. The application of defined cultures did control the open texture problem, however, and they were prone to slow acid production due to their susceptibility to bacteriophage. The use of pairs of phage-unrelated strains and culture rotation to prevent build up of phage in the cheese factory was practiced to minimize the potential for phage problems. Eventually, the use of multiple strain starter and factory-derived phage-resistant strains was made to control the phage problem.

Lactic starter cultures are also categorized based on flavour or gas production characteristics as follows:

- B or L cultures (*Betacoccus or Leuconostoc*) contain flavor and aroma producing organisms, for example, *Leuconostoc* spp.
- D cultures contain *Lactococcus diacetylactis*
- BD or DL cultures contain mixtures of both *Leuconostoc* and *S. diacetylactis* strains
- O cultures do not contain any flavor/aroma producers but contain *L. lactis* and *L. cremoris* strain.

**Table 1: Lactic starter cultures, associated microorganisms and their applications in the dairy industry**

<table>
<thead>
<tr>
<th>Lactic Acid Bacteria</th>
<th>Associated Microorganisms</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mesophilic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lactococcus lactis,</em></td>
<td><em>Lactococcus lactis</em> var. <em>diacetylactis,</em></td>
<td>Cheddar, Colby Cottage cheese, Cream cheese,</td>
</tr>
<tr>
<td><em>Lactococcus cremosis,</em></td>
<td><em>Penicillium camemberti,</em> <em>P. roqueforti,</em> <em>P. caseicolum,</em></td>
<td>Neufachatel, Camembert, Brie, Roquefort, Blue,</td>
</tr>
<tr>
<td><em>Lactococcus lactis</em> var.</td>
<td><em>Brevibacterium linens</em></td>
<td>Gorgonzola, Limburger</td>
</tr>
<tr>
<td><em>diacetylactis,</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Leuconostoc cremosis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Thermophilic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus thermophilus</em>,</td>
<td><em>Candida kefyr,</em> <em>Torulopsis,</em> <em>spp., L. brevis,</em></td>
<td>Parmesan, Romano, Grana Kefir, Koumiss yogurt,</td>
</tr>
<tr>
<td><em>Lactobacillus bulgaricus,</em></td>
<td><em>Bifidobacterium bifidum,</em> <em>Propionibacterium fureudenreichii,</em> <em>P. shermanii</em></td>
<td>Kefir, Koumiss yogurt, Yakult, Therapeutic cultured</td>
</tr>
<tr>
<td><em>L. lactis,</em> <em>L. casei,</em></td>
<td></td>
<td>milks, Swiss, Emmenthal, Gruyere</td>
</tr>
<tr>
<td><em>L. helveticus,</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. plantarum,</em> <em>Enterococcus faecium</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mixed starters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lactococcus lactis,</em></td>
<td>…</td>
<td>Modified Cheddar, Italian, Mozzarella, Pasta Filata,</td>
</tr>
<tr>
<td><em>S. thermophilus,</em></td>
<td></td>
<td>Pizza cheese</td>
</tr>
<tr>
<td><em>E. faecium,</em> <em>L. helveticus,</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. bulgaricus</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Starter cultures for fermented foods and beverages

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Hard cheeses without eyes</td>
<td><em>Lactococcus lactis</em> ssp. <em>lactis</em>, <em>L. lactis</em> ssp. <em>cremoris</em></td>
</tr>
<tr>
<td>3.</td>
<td>Swiss and Italian type cheeses</td>
<td><em>Lactobacillus delbrueckii</em> ssp. <em>lactis</em>, <em>L. helveticus</em>, <em>L. casei</em>, <em>L. delbrueckii</em> ssp. <em>bulgaricus</em>, <em>Streptococcus thermophilus</em></td>
</tr>
<tr>
<td>5.</td>
<td>Yoghurt</td>
<td><em>L. delbrueckii</em> ssp. <em>bulgaricus</em>, <em>Streptococcus thermophilus</em></td>
</tr>
<tr>
<td>6.</td>
<td>Fermented, probiotic milk</td>
<td><em>L. casei</em>, <em>L. acidophilus</em>, <em>L. rhamnosus</em>, <em>L. johnsonii</em>, <em>Bifidobacterium lactis</em>, <em>B. bifidum</em>, <em>B. brevis</em></td>
</tr>
<tr>
<td>7.</td>
<td>Kefir</td>
<td><em>L. kefir</em>, <em>L. kefiranofacies</em>, <em>L. brevis</em></td>
</tr>
<tr>
<td>8.</td>
<td>Kumiss</td>
<td></td>
</tr>
</tbody>
</table>

**Functional dairy starter cultures**

Milk contains many health promoting constituents including immunoglobulins, bioactive fatty acids and peptides etc. The healthy image of milk has resulted in dramatic growth in the diversification of dairy products in recent years and in huge increase in the varieties of products such as dairy desserts, flavoured milk drinks, cheeses, yoghurt etc. Apart from the milk components, the health attributes are associated with fermented and probiotic milks and dairy products. Milk has been preserved by fermentation through the action of lactic acid bacteria (LAB), which convert lactose to lactic acid and other organic acids, thereby lowering the pH and subsequently inhibiting the growth of pathogenic and spoilage bacteria. Moreover, these LAB produce a range of secondary metabolites, that can influence the products’ flavour, aroma and texture as well as antimicrobial peptides. These bacteria also possess a diverse complement of proteases and peptidases that aid in digestion of milk proteins. In addition, many bifidobacteria and lactobacilli are increasingly exploited in probiotic dairy products such as cheeses, yoghurt, milk drinks etc.

A novel trend in the food industry is to explore the use of functional starter cultures of LAB for the manufacture of fermented foods. Functional starter cultures are defined as starters that possess at least one inherent, functional property, aimed at improving the quality of the end product. Functional starter cultures of lactic acid bacteria are defined as strains that are used as starter cultures for food fermentation, and that are able to express certain functional properties which give an added value to the end-product (e.g. bacteriocin producers, exopolysaccharide producers, probiotic strains etc).
functionality can contribute to microbial safety, or offer one or more organoleptic, technological, or nutritional and health advantages to the food. Promising examples are LAB that produce antimicrobial substances such as bacteriocins to assure food safety, sugar polymers to improve texture, desirable aromatic compounds to enhance taste properties, or strains that display probiotic effects. To develop such cultures, the biodiversity of traditional fermented foods and spontaneous fermentation processes is analyzed.

**Metabolites produced by LAB**

LAB has relatively simple homo- or hetero- fermentative metabolism. These bacteria rely on lactose as their main carbohydrate source. Dairy LAB includes members of the genera Lacobacillus, Lactococcus, Leuconostoc and Sterptococcus. LAB fermentation yields primarily lactic acid, which plays a vital function in safeguarding food products. LAB metabolism beneficially affect the texture and flavour of fermented foods. The viscosity and texture of fermented dairy products can be greatly enhanced by the production of polysaccharides by LAB, while compounds such as diacetyl, ethanol, acetaldehyde, etc play vital roles in flavour development.

<table>
<thead>
<tr>
<th>Starter bacteria</th>
<th>Functionality</th>
<th>Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactococcus lactis ssp. lactis, L. lactis ssp. cremoris, Enterococcus spp., L. carvatus, L. sakei, P. acidilactici, E. faecium, L. plantarum, L. ruteri, Streptococcus thermophilus</td>
<td>Bacteriocins production</td>
<td>For bio-preservation of foods</td>
</tr>
<tr>
<td>Several EPS producing lactic acid bacteria (lactobacilli and streptococci, lactococci)</td>
<td>Exo-polysaccharides production</td>
<td>Good body and texture of low fat fermented dairy products</td>
</tr>
<tr>
<td>Galactose fermenting lactobacilli and streptococci</td>
<td>Good galactose utilization</td>
<td>Low level of galactose in fermented milk products</td>
</tr>
<tr>
<td>Lactose negative L. delbrueckii ssp. bulgaricus</td>
<td>Prevention of over acidification in yoghurt</td>
<td>Good body and texture</td>
</tr>
<tr>
<td>Autolysing lactic acid bacteria</td>
<td>Enhanced proteolytic and lipolytic activities</td>
<td>Acceleration ripening of cheeses</td>
</tr>
<tr>
<td>Mannitol, sorbitol producing lactic acid bacteria (Leuconostocs)</td>
<td>Production of low calorie sugars in fermented milks</td>
<td>Reduced calorie misthi dahi</td>
</tr>
</tbody>
</table>

**Table 3: Functional starter cultures and their applications**
<table>
<thead>
<tr>
<th>Probiotic lactic acid bacteria</th>
<th>Different functional attributes</th>
<th>Probiotic functional foods and dairy products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamins producing lactic acid bacteria (Streptococci) and propionibacteria</td>
<td>Improved the vitamin content in fermented dairy products</td>
<td>Good health to the consumers improve vitamin malnutrition</td>
</tr>
<tr>
<td>Raffinose, stachyose and verbascose utilizing lactic acid bacteria, phytic acid, tannins, cyanogenic glucosides degrading lactic acid bacteria</td>
<td>Removal/ reduction of toxic or antinutritive factors in cereal and legumes</td>
<td>Good health to the consumers for milk cereal, milk legumes fermented milk products improve nutrition</td>
</tr>
</tbody>
</table>

In addition, many LAB produce compounds of human nutritional value as regular end products in their metabolisms, including some B-vitamins. Many strains of LAB and bidifobacteria produce other metabolites that promote human health. Bioactive peptides generated from milk proteins as a results of their proteinase and peptidases activity and production of CLA from linoleic acid by strains of lactobacilli and bifidobacteria.

**Technology of starter cultures**

Lactic starter cultures are generally available from commercial manufacturers in spray-dried, freeze-dried (lyophilized), or frozen form. Spray-dried and lyophilized cultures need to be inoculated into milk or other suitable medium and propagated to the bulk volumes required for inoculating a cheese vat as follows:

![Starter Culture Flowchart]

Stock culture (Freze dried, frozen, spray dried) → Mother culture → Intermediate culture → Bulk culture → Process Milk (Multipurpose vat/Cheese Vat/Lassi tank)

Stock 1 ml 100 ml⁻¹ → Mother 1 ml 100 ml⁻¹ → Feeder 2 ml 100 ml⁻¹ → Bulk 200 liters

0.4 ml → 40 ml → 4 liters → 200 liters

However, the repeated sub-culturing of certain strains of starter bacteria may loss the plasmids and consequently can affect the characteristics (i.e., phage-resistant becomes phagesensitive, lack of lactose utilization etc). The yogurt starter cultures (S. thermophilus and L. delbrueckii ssp. bulgaricus)
are normally used the ratio of cocci : rods as 1 : 1. Starter culture activity is affected by the rate of cooling after incubation, level of acidity at the end of the incubation period, and the temperature and duration of storage. Many larger dairy plants develop their own cultures. However, preparing and maintaining bulk cultures requires specialized facilities and equipment. Much research and development in the starter culture technology has been aimed at designing specialized growth media for starters, protecting the starter cultures from sub-lethal stress and injury during freezing, and minimizing the threat of bacteriophage during starter culture preparations. Therefore, the use of concentrated direct vat starters is gaining much importance in preparation of fermented milks. The Direct Vat Starters (DVS) cultures are highly concentrated cultures that are made of mixtures of defined strains in predetermined proportions. The advantages of DVS are improved quality, high yield, less rejection of batches, ease of use and reliability.

**Starter concentrates**

Traditionally ‘bulk starter’ in liquid form was used to inoculate the milk used in the manufacture of cheese, yoghurt, buttermilk and other fermented products. Over the past 10-15 years, the use of starter cell concentrates designated as either Direct Vat Set (DVS) or Direct Vat Inoculation (DVI) cultures have increasing being used, particularly in small plants, to replace bulk starter in fermented dairy product manufacture. (Note that the terms DVI and DVS are used interchangeably although particular culture suppliers will tend to use only one term) Starter concentrates used in DVI cultures are concentrated cell preparations containing in the order of \(10^{11} \text{ to } 10^{13}\) CFU/g. They are available as frozen pellets (fig. 1) or in freeze-dried granular form (fig. 2).

**Commercial DVS frozen culture in pellet form**

Under normal conditions starter growth in milk results in a cell concentration of about \(10^9\) cfu/ml. Growth of starters in milk is limited by a number of factors including the accumulation of lactic acid. Concentrates can be produced by neutralisation (traditional fermentation technology) or removal of the lactic acid (using diffusion culture), recovering the cells by centrifugation, and by maintaining starter activity by freeze drying or freezing. Freeze-dried concentrates can be stored for some months at 4°C. Frozen concentrates are usually stored at -45°C or lower. Some suppliers recommend that their frozen DVI cultures are stored at -18°C.

**Production of starter concentrates**

Commercial starter cultures currently available for direct addition to production vats contain billions
of viable bacteria per gram, preserved in a form that could be readily and rapidly activated in the product mix to perform the functions necessary to transform the product mix to the desired cultured product. To attain that, the selected starter bacteria need to be grown in a suitable medium to high numbers and to concentrate the cells. The composition of the media used to grow various bacteria differs. Usually, the materials used in the growth media consist of food grade, agricultural by-products and their derivatives. The trade has special requirements for the raw materials that go into media formulations and for the way they are mixed and processed.

The generally used ingredients in media formulations include non-fat milk, whey, hydrolysates of milk and whey proteins, soy isolates, soy protein hydrolysates, meat hydrolysates and extracts, egg proteins, corn steep liquor, malt extracts, potato infusions, yeast extracts/yeast autolysates, sugars such as lactose, glucose, high-fructose corn syrup, corn sugar, sucrose, and minerals such as magnesium, manganese, calcium, iron, phosphates, salt, etc. For some fastidious bacteria, amino acids and vitamins may be included. The phosphates are added to provide mineral requirements as well as for buffering. For some bacteria, which need unsaturated fatty acids to protect cell membranes, trace quantities of polysorbates (Tweens) are added. To control foaming, food grade anti foam ingredients may be incorporated.

The medium is then either sterilized by heating at 121°C for a minimum of 15 minutes or heat-treated at 85-95°C for 45 minutes or subjected to ultrahigh temperature treatment (UHT) for a few seconds. After heat treatment, the medium is cooled to the incubation temperature. After the addition of the inoculum, the medium is incubated until the predetermined endpoint is reached. During incubation, the pH is maintained at a predetermined level (constant neutralization to maintain pH). Generally, the endpoint coincides with the exhaustion of sugar reflected by the trace of the neutralization curve. The frequency of neutralization reflects the activity of the culture in the fermenter, and when the frequency decreases, it indicates the near depletion of the sugar. Samples are usually taken to microscopically examine the fermentate for cell morphology, for any gross contamination, for a rough estimation of cell numbers, and for quantitative measurement of sugar content. After ascertaining these, the fermenter is cooled. The cells are harvested either by centrifugation or by ultrafiltration. The cell concentrate is obtained in the form of a thick liquid of the consistency of cream and is weighed and rapidly cooled. Sterile preparations of cryoprotectants (glycerol, nonfat milk, monosodium glutamate, sugars, etc.) are added, and uniformly mixed with the cell concentrate. The concentrate may be filled as such into cans and frozen or frozen in droplet form in liquid nitrogen (pellets), retrieved, and packaged. The concentrate as such or in pellet form may also be lyophilized in industrial scale freeze dryers.

**pH control systems**

There are two main reasons for using pH control systems in propagating bulk starter cultures:

1. To minimize daily fluctuations in acid development and thereby prevent "over-ripening" of the starter.
2. To prevent the cellular injury that may occur to some starters when the pH of the medium drops below 5.0.

In the pH control systems, the acid produced by the starter culture is neutralized to maintain the pH at around 6.0. The external pH control system, uses whey based medium fortified with phosphates and yeast extract. The pH is maintained at around 6.0, by intermittent injection of anhydrous or aqueous ammonia, or sodium hydroxide. This system has been used successfully in the United States for production of most American-style cheeses. The internal pH control system, developed uses a whey based medium containing encapsulated citrate-phosphate buffers that maintain the pH at around 5.2. Unlike in the external pH control system, no addition of ammonia or NaOH is necessary.

**Phage inhibitory and phage-resistant medium (PIM/PRM)**
The PIM/PRM were developed following observations of Reiter64 that bacteriophage of lactic streptococci were inhibited in a milk medium lacking in calcium. Hargrove364 reported on the use of phosphates to sequester free calcium ions in milk or bulk-starter medium for inhibition of bacteriophage. The effectiveness of phosphates in the formation of PIM/PRM for phage control was confirmed by Christensen. The PIM/PRM consisting mainly of milk solids, sugar, buffering agents such as phosphates and citrates and yeast extract have been widely used in the United States, Canada, and Europe for about 20 years. However, the effectiveness of the PIM/PRM in inhibiting bacteriophage and stimulating growth of the starter culture media is somewhat limited. Despite the absence of calcium, some phages can infect the the starter culture at its optimum growth temperature. Also, phosphates in the PIM/PRM can cause metabolic injury to some starter cultures. The preparation of active bulk starter culture free of phage contamination is essential for cheese manufacturing. If the pH is maintained in the region 6.0 - 6.3 by neutralisation of the lactic acid produced by the starter bacteria then the cell population can be increased about 10-100 fold depending on the neutraliser used. Both sodium hydroxide and ammonium hydroxide have been used, use of the latter results in higher cell densities. The cessation of growth of starters grown in fermentation media under pH control is due to several factors including the accumulation of inhibitory concentrations of lactate, hydrogen peroxide, nisin, D-leucine.

Higher cell densities (greater than \(10^{10}\) CFU/g) can be obtained by harvesting the cells from the fermenter medium by centrifugation, to give a starter population of \(10^{11} - 10^{12}\) CFU/ml. Even higher cell densities can be obtained by freeze drying the 'sludge' obtained by centrifugation. Unfortunately, the increase in cell population for some strains does not necessarily parallel the increase in the ability of the concentrated culture to produce acid. These strains are susceptible to damage during the fermentation, centrifugation and freeze-drying/freezing and storage stages.

**Advantages of using cell concentrates**

The following advantages have been claimed for the use of concentrates in cheese factories:

- Centralised concentrate production enables a manufacturer to establish a team of technical experts and to develop the necessary technology and protocols to produce a quality product.
- Concentrates can be produced at a central site, which is located at a place distant from cheese manufacture thus avoiding the hazards of phage infection due to phage-leaden whey aerosol particles in the environment.
- Detailed quality control tests can be performed on each batch of concentrate and, in theory at least, only batches meeting the manufacturer's specification are released for factory use.
- No incubation or sub-culturing is required at the factory. This reduces the probability of phage or other forms of contamination occurring since all the factory-staff have to do is to thaw the concentrate or open a packet of freeze-dried concentrates and add it to the bulk starter milk or to the vat milk.

**Disadvantages of using starter concentrates**

The following disadvantages have been claimed for the use of concentrates:

- Not all starters respond well to the operations involved in concentrate production and/or storage. This fact limits the number of strains suitable for concentrate production and can create some difficulties for starter suppliers and factory laboratories in compiling rotations of phage unrelated strains. This reduction in the numbers of strains also results in a more limited choice of starters that have the potential to produce good flavour in mature cheese. To some extent this has
prompted the development of adjunct cultures, some of which may be used to enhance or even balance flavour in mature cheese.

- Low temperature storage facilities are required for frozen concentrates at the production point, during transit to the factory and at the factory. Power cuts and distribution problems could obviously present difficulties. Some of these difficulties have been overcome by the development of freeze-dried concentrates.

- Although concentrate suppliers perform quality assurance on their products, starter suppliers generally offer only limited guarantees of concentrate quality. In other words, if a contaminated concentrate is used and an inferior quality cheese results, or worse, there may be difficulties in getting the starter supplier to accept liability for all the resultant economic loss. In fact, most starter suppliers recommend that concentrates should be pre-tested at the factory before their use in cheese manufacture. Consequently, a decision to replace the mother and the intermediate stages of bulk starter manufacture with concentrates should be based on the knowledge that the responsibility for starter quality has been taken from the factory laboratory and belongs to the starter supplier. However, the accountability in the event of problems related to the starter may not have been fully transferred to the supplier. For these reasons factories using concentrates should ideally take representative samples from each batch of concentrates and pre-test them before cheese and other ferment products manufacture. Factories lacking the facilities to do this should take samples and in the event of problems and send concentrate samples unopened, packaged properly and refrigerated to an independent laboratory for analysis.

- Use of DVS cultures is expensive compared with bulk starter manufacture. This is particularly so when the costs of modern, aseptically produced starter using pH control are considered. The costs are well understood but this statement is only valid where companies have well designed bulk starter facilities, qualified staff and good quality assurance laboratories. In the absence of this combination, the economic losses resulting from poor-quality cheese, means that use of DVI cultures is the logical choice for many small to medium sized processing units.

- Changes to the cheese making process may be required. Addition of traditional bulk starter to cheese milk results in a drop of 0.1 to 0.2 pH units. This small drop in acidity has significant even if subtle effects on subsequent proteolysis in the cheese, and has an effect on coagulation. In addition, the culture starts producing acid virtually immediately. With DVS cultures, there is no drop in acidity and there is a lag period before the cultures commences growth and acid production. Consequently, small adjustments to the traditional cheese making process are required to maintain cheese quality.

### Table 3: Storage conditions and shelf lives of some concentrated cultures

<table>
<thead>
<tr>
<th>Type of cultures</th>
<th>Storage</th>
<th>Shelf-life (Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Freeze dried (Direct Vat)</td>
<td>-18°C</td>
<td>12</td>
</tr>
<tr>
<td>2. Deep frozen (Direct vat)</td>
<td>-45°C</td>
<td>12</td>
</tr>
<tr>
<td>3. Freeze dried (Master culture)</td>
<td>+5°C</td>
<td>12</td>
</tr>
</tbody>
</table>

**Quality control of commercial cultures (DVS/DVI)**

1) Viable cell numbers
2) Absence of contaminants, pathogens, and extraneous matter
3) Acid-producing and other functional activities
4) Package integrity, accuracy of label information on the package
5) Shelf life of the product according to specification

<table>
<thead>
<tr>
<th>Starter organism</th>
<th>10^{10} - 10^{12} cfu/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliforms</td>
<td>Absence in 1 g</td>
</tr>
<tr>
<td>Enterococci</td>
<td>Less than 20 cfu/g</td>
</tr>
<tr>
<td>Yeasts and molds</td>
<td>Absence in 1 g</td>
</tr>
<tr>
<td>Staphylococci (coagulase-positive)</td>
<td>Absence in 10 g</td>
</tr>
<tr>
<td>Listeria</td>
<td>Absence in 25 g</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Absence in 25 g</td>
</tr>
</tbody>
</table>

Conclusion
Commercial starter culture production is a highly demanding operation. It requires specialized knowledge of microbiology, microbial physiology, process engineering, and cryobiology. In addition to production knowledge, a full-fledged quality control program is necessary to test incoming raw materials, design and maintain plant sanitation, test sterility of production contact surfaces, monitor plant environment quality, and test every product lot for the prescribed quality standards. The quality control section is also required to train and update plant personnel on the importance of sanitation and strict adherence to process control protocols.

Suggested readings


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Introduction

Foods which promote health beyond providing basic nutrition are termed as ‘functional foods’. The term ‘functional food’ refers to a food that has been modified or value-added. Significant strategy in the development of functional foods evolves increasing the levels of specific nutraceuticals that are known to have health benefits. This can be through enhancement of levels of the desired component that is inherent in the food or by fortification of food products with functional ingredients, such as dietary fibres, antioxidants, natural isoflavones, plant sterols/stanols, other phytochemicals or phytoneutrients, bioactive peptides, ω-3, ω-6 PUFA, probiotics, prebiotics, minerals and vitamins etc (Table 1).

Table 1. Functional ingredients and the health benefits

<table>
<thead>
<tr>
<th>Functional ingredients addition/modifications of foods</th>
<th>Functionality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytochemicals (as plant ingredients or extracts)</td>
<td>Antioxidant, lower risk of CHD, cancer, and lower blood pressure</td>
</tr>
<tr>
<td>Probiotics</td>
<td>Improved gastrointestinal function, enhanced immune system, lower risk of colon cancer and of food allergy</td>
</tr>
<tr>
<td>Prebiotics</td>
<td>Improved gastrointestinal function, lower risk of colon cancer, enhanced immune system</td>
</tr>
<tr>
<td>Bioactive proteins or peptides</td>
<td>Enhanced immune function and bioavailability of minerals, hypertensive function</td>
</tr>
<tr>
<td>Dietary fibers</td>
<td>Prevention of constipation, lower risk of colon cancer and lowering of blood cholesterol level</td>
</tr>
<tr>
<td>ω-3 PUFA</td>
<td>Lower risk of heart attack, lower risk of some cancers, enhanced immune system</td>
</tr>
<tr>
<td>Removal of allergens</td>
<td>Reduce or eliminate allergy to specific foods</td>
</tr>
<tr>
<td>Hydrolysis of lactose by adding β-galaactosidase</td>
<td>Enable digestion of lactose by lactose-intolerant persons</td>
</tr>
</tbody>
</table>

Functional food ingredients should be present in sufficient quantities/numbers at the time of consumption and reach to the action site for functional activities. Thus, the ingredients should withstand the food processing and preservation treatments and stable during storage and gastrointestinal tract (GIT) transient. These should not be affected by food matrixes and environmental factors prevailed in foods as well as should not react with food components. However, most of the ingredients react with food components and also affected by food matrixes and
environments. These lead to the poor stability/survival of functional ingredients. Different methods for stabilization or improvement of survival are including selection of suitable and stable ingredients, food combinations, addition of protective agents, segregation by physical barriers etc. The selection is highly probabilistic in nature. Alternatively, addition of compatible protective/stabilizing agents and segregation by applying barriers are very suitable and promising. Controlled release of food ingredients at the right place and the right time is the key for the functionality of active ingredients. A timely and targeted release improves the effectiveness of food additives, broadens the application range of ingredients and ensures optimal dosage and cost-effectiveness. Among the different techniques, microencapsulation offers advantages in improving the nutrient content of foods without affecting the sensory qualities. Microencapsulation may is used for stabilizing a desirable component, reducing the level of an undesirable component and enabling the targeted delivery of functional ingredients.

**Hurdles affecting the functional food ingredients**

**Long chain poly-unsaturated fatty acids**

Numerous challenges exist in the production, transportation and storage of poly-unsaturated fatty acids (such as ω-3, ω-6 fatty acids) fortified foods as poly-unsaturated fatty acids are extremely susceptible to oxidative deterioration. It has been a challenge for oil refiners to inhibit oxidation ω-3 fatty acids during processing, shipping, and storage. Additional challenges exist in preventing the oxidation of ω-3 fatty acids when these are incorporated into processed foods.

**Vitamin and minerals**

Vitamin and mineral fortification has been used to improve nutrient content of foods. The level of vitamins decreases during processing and storage. The interactions between the added minerals and vitamins with other components in foods are important for fortification. pH, heat, light, oxygen, oxidizing agents and enzymes decrease the stability and activity of many vitamins. The addition of free mineral salts is having undesirable interactions between mineral salts and components in milk and milk products can lead to precipitation, colour and flavour problems, and the bio-availability. The fortification of milk with iron presents different challenges. The most common iron salts (e.g. ferrous sulphate). The addition of these salts can affect the sensory properties of the food due to the taste of the iron salt or the catalytic effect of iron the oxidaiton of fats. Some of the major problems encountered by direct addition of simple calcium salts, such as loss of heat stability due to increase in calcium activity can be overcome by using calcium complexing agents or insoluble calcium salts.

**Probiotics**

Probiotics are added as live cultures in a range of foods to improve the microbial balance of the human gut. The survival of probiotics in foods during processing, preservation and storage as well as during GIT transient is the determinant of probiotic functional foods. The consumption of sufficient viable cells ($10^8$-$10^9$ cfu/day) is required for functional activities. However, probiotics survive poor in traditional fermented dairy products due to low pH, post-acidification (during storage), hydrogen peroxide production, oxygen toxicity, storage temperatures, poor growth in milk and lack of compatibility with traditional starter cultures, etc. and during GIT transient. For increasing probiotic consumption, foods with probiotics need to be diversified to non-fermented, where probiotics survival and compatibility is a big impediment.
Microencapsulation an efficient delivery system

In microencapsulation droplets/particles of liquids, solids, or gases (core) are coated by thin films (coatings), which protect the core from external environment. The core can be released at different times as and when required by any desired mechanisms, such as disruption, dissociation, dissolution or diffusion and with any desired rates. The coating on a core is semi-permeable and protects the core from severe conditions and controls substances flowing into the core and the release of metabolites from the core. Encapsulation in foods is also utilized to mask odours or tastes. Various techniques are employed to form the capsules, including spray drying, spray chilling or spray cooling, extrusion coating, fluidized bed coating, liposome entrapment, coacervation, inclusion complexation, centrifugal extrusion and rotational suspension separation (Table 2). Number of food ingredients/substances have been microencapsulated, such as acidulants, amino acids, antimicrobials, bases, colorants, edible oils, flavour, enzymes, microorganisms, flavour enhancers, leavening agents, minerals, sugars, salts, vitamins etc. The use of encapsulation for sweeteners such as aspartame and flavours in chewing gum is well known. Fats, starches, dextrins, alginites, protein and lipid materials can be employed as encapsulating materials. Various methods exist to release the ingredients from the capsules such as site-specific, stage-specific or signalled by changes in pH, temperature, irradiation or osmotic shock. In the food industry, the most common method is by solvent-activated release. The addition of water to dry beverages or cake mixes is an example. Liposomes have been applied in cheese-making, and its use in the preparation of food emulsions such as spreads, margarine and mayonnaise is a developing area. Most recent developments include the encapsulation of foods in the areas of controlled release, carrier materials, preparation methods and sweetener immobilization. New markets are being developed and current research is underway to reduce the high production costs and lack of food-grade materials.

Table 2: Techniques for microencapsulation

<table>
<thead>
<tr>
<th>Technique</th>
<th>Major steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Spray-drying</td>
<td>a. Preparation of the dispersion</td>
</tr>
<tr>
<td></td>
<td>b. Homogenization of the dispersion</td>
</tr>
<tr>
<td></td>
<td>c. Atomization of the infeed dispersion</td>
</tr>
<tr>
<td></td>
<td>d. Dehydration of the atomized particles</td>
</tr>
<tr>
<td>2. Spray-cooling</td>
<td>a. Preparation of the dispersion</td>
</tr>
<tr>
<td></td>
<td>b. Homogenization of the dispersion</td>
</tr>
<tr>
<td></td>
<td>c. Atomization of the infeed dispersion</td>
</tr>
<tr>
<td>3. Spray-chilling</td>
<td>a. Preparation of the dispersion</td>
</tr>
<tr>
<td></td>
<td>b. Homogenization of the dispersion</td>
</tr>
<tr>
<td></td>
<td>c. Atomization of the infeed dispersion</td>
</tr>
<tr>
<td>4. Fluidized-bed coating</td>
<td>a. Preparation of coating solution</td>
</tr>
<tr>
<td></td>
<td>b. Fluidization of core particles</td>
</tr>
<tr>
<td>Process</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
</tbody>
</table>
| 5. Extrusion | a. Preparation of molten coating solution  
b. Dispersion of core into molten polymer  
c. Cooling or passing of core-coat mixture through dehydrating liquid |
| 6. Centrifugal extrusion | a. Preparation of core solution  
b. Preparation of coating material solution  
c. Co-extrusion of core and coat solution through nozzles |
| 7. Lyophilization | a. Mixing of core in a coating solution  
b. Freeze-drying of the mixture |
| 8. Coacervation | a. Formation of a three-immiscible chemical phases  
b. Deposition of the coating  
c. Solidification of the coating |
| 9. Centrifugal suspension separation | a. Mixing of core in a coating material  
b. Pour the mixture over a rotating disc to obtain encapsulated tiny particles  
c. Drying |
| 10. Co-crystallization | a. Preparation of supersaturated sucrose solution  
b. Adding of core into supersaturated solution  
c. Emission of substantial heat after solution reaches the sucrose crystallization temperature |
| 11. Liposome entrapment | a. Micro-fluidization  
b. Ultrasonication  
c. Reverse-phase evaporation |
| 12. Inclusion complexation | Preparation of complexes by mixing or grinding or spray-drying |

**Consideration of materials for micro-encapsulation**

The structure formed by microencapsulating agent around the core material is called the wall material, which protects the core against deterioration, limits the evaporation of volatile core materials. The encapsulating agents should have certain ideal characteristics, depending on the objectives and requirements, process of encapsulation, chemical characteristics of the core material, the intended use of the core material, the conditions under which the product will be stored, and the processing
conditions to which it will be exposed. Some general characteristics of the encapsulating agent are that it is insoluble in and non-reactive with the core material, have solubility in the end-product food system, and be able to withstand high temperature processing. Some typical encapsulation agents are dextrans, gums, starches or proteins (Table 3). Many coating materials have been used for encapsulation of microorganisms. These include a mixture of κ-carrageenan and locust bean gum, cellulose acetate phthalate, alginate, alginate-starch mixture, κ-carrageenan etc.

Table 3: Coating materials for encapsulation

<table>
<thead>
<tr>
<th>Class of coating materials</th>
<th>Specific types of coatings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gums</td>
<td>Gum arabic, agar, sodium alginate, carrageenan</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Starch, dextran, sucrose, corn syrup</td>
</tr>
<tr>
<td>Celluloses</td>
<td>CMC, methylcellulose, ethylcellulose, nitrocellulose, acetylcellulose, cellulose acetate-phthalate, cellulose acetate-butylate-phthalate</td>
</tr>
<tr>
<td>Lipids</td>
<td>Wax, paraffin, tristearin, stearic acid, monoglycerides, diglycerides, beeswax, oils, fats, hardened oils</td>
</tr>
<tr>
<td>Inorganic materials</td>
<td>Calcium sulfate, silicates, clays</td>
</tr>
<tr>
<td>Proteins</td>
<td>Gluten, casein, gelatin, albumin</td>
</tr>
</tbody>
</table>

Additional treatments to microcapsules

Entrapment in hydrocolloid gels, such as alginate, κ-carrageenan etc have some limitations due to less stability of microcapsules in the presence of chelating agents such as phosphate, lactate, citrate etc., which share the affinity for ions such as Ca$^{+2}$, K$^+$, etc. and destabilize the gel. The problems are encountered during lactic acid fermentation and cause cell release from the beads. In other matrix material, such as chitosan, the entrapped cells can be released from the beads during fermentation and cause low initial loading for the next fermentation. Therefore, additional treatments, such as coating the beads, are applied to improve the properties of beads. Coated beads not only prevent cell release but also increase mechanical and chemical stability. Cross-linking with cationic polymers, coating with other polymers, mixing with starch and incorporating additives improve stability of beads.

Applications

Protection of polyunsaturated fatty acids

Microencapsulation of long-chain polyunsaturated oils eliminates fishy odour and taste for development of enriched fatty acids products. A supplement comprising a blend of omega-3 fatty acids, omega-6 fatty acids (gama-linoleic acid C18: 3n-6 and arachidonic acid C20:4n-6) and evening primrose oil encapsulated in gelatine may be provided for addition to infant formulae to achieve a milk composition approximating to human milk. Infant formulae fortified with microencapsulated spray dried marine oil powders have been successful in the market place. Yoghurts, fermented milks and processed cheese with tuna oil encapsulated with processed milk-
protein-carbohydrate films (Driphorm 50) made using MicroMAX technology have higher sensory scores than those fortified with an equivalent amount of non-encapsulated oil. The development of spray-dried microencapsulated fish oil was undertaken as part of EU FAIR contact 9CT 95-0085 to establish a delivery system for fish oil in powdered form so that there would be a degree of protection from oxidation during storage, containment of fishy odour as far as possible, and finally to achieve the highest oil content possible in dry matter. Deodorized sand-ell oil (fish oil) stabilized with natural antioxidants was emulsified with protein and lactose (oil: protein: lactose: water in 10:10:10:70 ratio). Both the processing variables (homogenization pressure and number of passes, and spray-drying effects) and packaging (vacuum vs. nitrogen flushing) were studied. Based on physical indicator, it was concluded that homogenization pressure and protein source (sodium caseinate, calcium caseinate, and skim milk powder) influenced free fat and surface fat contents in the powder. Skim milk powder gave better sensory scores. The resulting microencapsulated fish oil powder had very good sensory properties and was stable for up to 6 months under refrigerated conditions.

**Protection of vitamins and minerals**

Many encapsulated preparations for addition to a range of beverage and foods have been developed to overcome undesirable interaction of vitamins with the environment and food components during processing and storage. Microencapsulated vitamins improve the stability of vitamins' stability during storage. Higher levels of the added vitamin D are entrapped into the cheese curd when milk is fortified with liposome encapsulated vitamin as compared to homogenizing in cream or milk (Table 4).

Table 4: Stability of vitamin preparations in dairy products

<table>
<thead>
<tr>
<th>Product</th>
<th>Type of vitamin preparation</th>
<th>% Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instant skim milk powder</td>
<td>Gelatin-encapsulated vitamin A (after simulated instantising treatment)</td>
<td>60% in 40 weeks of storage</td>
</tr>
<tr>
<td></td>
<td>Gelatin-encapsulated vitamin A (Dry blends with non-fat dry milk)</td>
<td>Approx. 10% in 40 weeks storage</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>Water-soluble emulsion of vitamin D</td>
<td>16% in 7 months</td>
</tr>
<tr>
<td></td>
<td>Vitamin D homogenized in cream</td>
<td>11% in 7 months</td>
</tr>
<tr>
<td></td>
<td>Vitamin D entrapped in liposomes</td>
<td>40% in 7 months</td>
</tr>
</tbody>
</table>

Encapsulated mineral salts lessen the tendencies of undesirable interactions. The choice between fortification with microencapsulated minerals or direct addition of mineral salts is depend on their relative costs, the bioavailability and impact on sensory properties of foods. Microencapsulated iron ingredients can prevent off-flavour development and maintain bioavailability of the iron. Stearine-coated iron salts decrease fat oxidation in Harvati cheese compared to unprotected iron salt. Liposome encapsulated iron may be used for fortification of beverages for minimizing off-flavours and interactions with other food components. The use of ferrous sulphate encapsulated in lecithin (SFE-171, Biofer) is claimed to allowed effective fortification of fluid milk and dairy products, while preventing undesirable interactions with milk components with higher bio-availability of iron. Iron absorption from milk with the use of encapsulated ferrous sulphate SFE-171 is higher than that with
the direct addition of ferrous sulphate. A possible strategy for calcium fortification of fluid milk includes the use of micro-crystalline cellulose-based ingredient co-processed with calcium carbonate and carboxymethylcellulose, which results in good flavour and stability of milk. Alternatively, liposomes may be used to protect calcium salts from interactions with proteins at higher temperature, as these prevent precipitation of soy proteins in calcium-fortified soy milk.

**Protection of probiotics**

Viability of probiotics can be improved by appropriate selection of acid and bile resistant strains, use of oxygen impermeable containers, two-step fermentation, stress adaptation, incorporation of micronutrients such as peptides and amino acids, sonication of yogurt bacteria and microencapsulation. Microencapsulation is the most suitable alternative technology to offer the best protection to the probiotic cells resulting from the freeze-drying and milling and such microencapsulated probiotics can be used in numerous food systems (Table 5 and 6).

**Table 5: Food and biotechnological applications of encapsulated bacteria**

<table>
<thead>
<tr>
<th>Cultures</th>
<th>Encapsulating materials</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. bifidum, B. infantis</em></td>
<td>Calcium alginate</td>
<td>Mayonnaise</td>
</tr>
<tr>
<td><em>L. paracasei</em></td>
<td>Milk fat</td>
<td>Cheddar cheese</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>Milk fat</td>
<td>Cheddar cheese</td>
</tr>
<tr>
<td><em>B. bifidum, B. adolescentis</em></td>
<td>Cream</td>
<td>White brined cheese</td>
</tr>
<tr>
<td><em>B. bifidum, B. infantis, and B. longum</em></td>
<td>Calcium alginate gels</td>
<td>Crescenza cheese</td>
</tr>
<tr>
<td><em>L. lactis subspp. Lactis</em></td>
<td>k-Carrageenan and locust bean gum</td>
<td>Fresh cheese</td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td>Liquid core alginate capsule</td>
<td>Lactic acid</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>Calcium alginate</td>
<td>Frozen dessert</td>
</tr>
<tr>
<td><em>Lactococci</em></td>
<td>Calcium alginate</td>
<td>Cream</td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td>k-Carrageenan and locust bean gum</td>
<td>Yoghurt</td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td>Calcium alginate</td>
<td>Milk chocolate, kulfi</td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td>Skim milk-whey protein concentrate-maltodextrin</td>
<td>Kulfi</td>
</tr>
</tbody>
</table>

**Sustained and target release of functional ingredients**

Immunoglobulins have potential in functional food development as they afford protection against gastrointestinal infections. However, they are prone to inactivation in the gut. Encapsulation of immunoglobulins may be used to preserve the activity in certain environments. Milk immunoglobulin G (IgG) that was encapsulated in double emulsions, solid agar or calcium alginate gels had improved
stability in acid and alkali environments as well as making them less susceptible towards the action of proteinases. Sustained release of amino acids after ingestion of protein supplements is desirable in sports nutrition and for improved exercise performance. Liposomal encapsulated ion-exchange whey protein as a protein supplement maintains plasma amino acids at higher levels compared to when conventional proteins supplements. Liposome encapsulated cholesterol-lowering plant sterols and stanols are used in milk and dairy products and these preparations are alternative to the free stanols and sterols having limited solubility in some foods.

### Table 6: Survival of encapsulated probiotics in milk and milk products

<table>
<thead>
<tr>
<th>Products</th>
<th>Storage conditions</th>
<th>Form of bacteria added</th>
<th>Initial</th>
<th>After storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free B. bifidum</td>
<td></td>
<td></td>
<td>4.5x10⁶</td>
<td>7.5x10⁶</td>
</tr>
<tr>
<td>Yogurt</td>
<td>30 days at 4.4°C</td>
<td>Free B. longum B6</td>
<td>1.51x10⁹</td>
<td>3.54x10⁸</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. longum B6 enapsulated in k-carrageenan</td>
<td>1.51x10⁹</td>
<td>1.02x10⁹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Free B. longum ATCC 15708</td>
<td>1.51x10⁹</td>
<td>4.35x10⁸</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. longum ATCC 15708 enapsulated in K-carrageenan</td>
<td>1.51x10⁹</td>
<td>1.48x10⁹</td>
</tr>
<tr>
<td>Milk with 2% fat</td>
<td>12 days at 4°C</td>
<td>Free B. longum Bb-46</td>
<td>1.00x10⁷</td>
<td>1.00x10⁵</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. longum Bb-46 encapsulated in Ca alginate</td>
<td>4.00x10⁷</td>
<td>3.00x10⁵</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Free B. lactis Bb-12</td>
<td>1x10⁷</td>
<td>1x10⁷</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. lactis Bb-12 encapsulated in Ca alginate</td>
<td>2x10⁸</td>
<td>2x10⁸</td>
</tr>
<tr>
<td>Milk chocolate</td>
<td>60 days at 7°C</td>
<td>Free L. casei NCDC 298</td>
<td>3.4x10⁸</td>
<td>4.6x10⁸</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. casei NCDC 298 encapsulated in Ca alginate</td>
<td>4.4x10⁸</td>
<td>4.2x10⁸</td>
</tr>
<tr>
<td></td>
<td>30 days at room temperature</td>
<td>Free L. casei NCDC 298</td>
<td>5.3x10⁸</td>
<td>6.2x10⁵</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. casei NCDC 298 encapsulated in Ca alginate</td>
<td>3.9x10⁸</td>
<td>7.2x10⁶</td>
</tr>
<tr>
<td>Kulfi</td>
<td>-20°C for 7 days</td>
<td>L. casei NCDC 298 encapsulated in skim milk – whey protein concentrate-</td>
<td>2.0x10⁷</td>
<td>3.210⁷</td>
</tr>
<tr>
<td>Temperature</td>
<td>Ingredient</td>
<td>Microencapsulated in</td>
<td>CFU/g</td>
<td>CFU/g</td>
</tr>
<tr>
<td>-------------</td>
<td>------------</td>
<td>----------------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>-20°C for 35 days</td>
<td>L. casei NCDC 298</td>
<td>Ca-alginate</td>
<td>3.6x10^8</td>
<td>4.1x10^8</td>
</tr>
</tbody>
</table>

**Conclusion**

Fine-tuned controlled release and stabilization of functional ingredients in foods and during GIT transient is the key for development of functional foods. Among the different techniques, microencapsulation is no longer just an added value technique, but the source of totally new ingredients with matchless properties and can be applied in the development of new and novel functional foods. It is only one of a suite of technologies that may be applied to enhance the quality of healthy dairy foods and its suitability depends on the food product to be fortified, the need for protection of food components and timed release of nutraceuticals.

**Suggested references**


Introduction

The primary role of diet is to provide sufficient nutrients to meet the nutritional requirements of an individual. There is now increasing scientific evidence to support the hypothesis that some foods and food components have beneficial physiological and psychological effects over and above the provision of the basic nutrients. Many traditional food products including fruits, vegetables, soya, whole grains and milk have been found to contain components with potential health benefits and are referred as “Functional foods”. Today, the identification of biologically active components in foods that have the potential to optimize physical and mental wellbeing along with therapeutic attributes is of increasing importance. Indeed several food components, dairy products in particular owing to their wide reach, have purported a variety of health and nutritional benefits in recent years. These claims based on food and dietary supplement generally fall into three categories: health claims, nutrient content claims, and structure/function claims. The responsibility for ensuring the validity of these claims rests with the manufacturer or drug administration body of the country concerned.

Health claims

Health claims describe a relationship between a food, food component, or dietary supplement ingredient, and reducing risk of a disease or health-related condition. A “health claim” by definition has two essential components: (1) a substance (whether a food, food component, or dietary ingredient) and (2) a disease or health-related condition. A statement lacking either one of these components does not meet the regulatory definition of a health claim. For example, statements that address a role of dietary patterns or of general categories of foods (e.g., fruits and vegetables) in health are considered to be dietary guidance rather than health claims, provided that the context of the statement does not suggest that a specific substance is the subject.

Nutrient content claims

The Nutrition Labeling and Education Act of 1990 (NLEA) permits the use of label claims that characterize the level of a nutrient in a food (i.e., nutrient content claims) made in accordance with FDA’s authorizing regulations. Nutrient content claims describe the level of a nutrient or dietary substance in the product, using terms such as free, high, and low, or they compare the level of a nutrient in a food to that of another food, using terms such as more, reduced, and lite. An accurate quantitative statement (e.g., 200 mg of sodium) that does not "characterize" the nutrient level may be used to describe any amount of a nutrient present. However, a statement such as "only 200 mg of sodium" characterizes the level of sodium as being low and would therefore need to conform to the criteria of an appropriate nutrient content claim or carry a disclosure statement that it does not comply with the claim. The requirements that govern the use of nutrient content claims help ensure that descriptive terms, such as high, low, or moderate, are used consistently for all types of food products and are thus meaningful to consumers. Healthy has been defined by a regulation as an implied nutrient content claim that characterizes a food that has "healthy" levels of total fat, saturated fat, cholesterol and sodium. Percentage claims for dietary supplements are another category of nutrient content claims. These claims are used to describe a percentage level of a dietary ingredient for which there is no...
established Daily Value. Examples include simple percentage statements such as "40% omega-3 fatty acids, 10 mg per capsule," and comparative percentage claims, e.g., "twice the omega-3 fatty acids per capsule (80 mg) as in 100 mg of menhaden oil (40 mg)."

Structure/Function claims

Structure/function claims describe the role of a nutrient or dietary ingredient intended to affect normal structure or function in humans, for example, "calcium builds strong bones." In addition, they may characterize the means by which a nutrient or dietary ingredient acts to maintain such structure or function, for example, "fiber maintains bowel regularity," or "antioxidants maintain cell integrity," or they may describe general well-being from consumption of a nutrient or dietary ingredient. Structure/function claims may also describe a benefit related to a nutrient deficiency disease (like vitamin C and scurvy), as long as the statement also tells how widespread such a disease is in the United States. The manufacturer is responsible for ensuring the accuracy and truthfulness of these claims; they are not pre-approved by FDA but must be truthful and not misleading.

Scientific validation of functional foods claims

A variety of natural products containing health claims are available commercially and many more are in pursuit of being commercialized. In this scenario, the consumer needs proper awareness of the purported claims and this has warranted stringent techniques for validation of effects of functional foods.

Conventional animal trials

Animal studies have long formed the basis of endorsement of various claims of functional foods. Laboratory animals have organs and body systems similar to humans and other animals and are generally susceptible to the same conditions and syndromes that affect humans. Because of this, the data derived from therapeutic research involving functional foods on these animals can be applied directly to humans and to other animals as well. Further, a wide range of specific animal strains have been developed which offer unique advantage of focusing in research matter of interest. For example, rats and rabbits are among best models for studying cardiovascular diseases while mice is favored for immunological analysis and germ free mice are available for probiotic research and other animal research requiring careful control of outside contaminants that can affect the experiment. Indeed, a vast majority of nutritional health claims of functional foods are derived from animal studies. Despite these, there are several limitations of animal studies in terms of interspecies difference between humans and animals. Body size, for example, affects biokinetics and oxidative stress; different species may differ with regard to metabolic enzymes, brain size, developmental speed, and the development of different cancer types—to name a few. Thus as vital as animals have been in characterizing effects of functional foods; they cannot be treated as sole parameters for validating functional foods.

In vitro and ex-vivo trials

In vitro (test tube or “in glass”) research and human cell cultures have proven superior to animal tests for a multitude of purposes. The primary advantage of in vitro research is that it permits simplification of the system or disease under study, allowing the investigator to focus on a small number of components. This becomes important in validating claims of functional foods in specific areas such as intestinal functions, diseases preventing investigations and nutritional assessments. Indeed, a vast variety of cell lines representing different cells and tissues are today available for in vitro measurements. These include human cell lines (HeLa, MCF-7, THP-1, Saos-2 cells, Caco-2 etc) and mouse cell lines (OCD, P19 etc). The in vitro techniques can also be used to predict in vivo performance of functional food components and thus reduce unnecessary human studies, accelerate
product development, and hasten evaluation of post-approval change. Despite these benefits, in vitro assessment of functional foods has several limitations. The in vitro model may not necessarily reflect all the physiological effects and changes in the desired system due to lack of complex interactions in physiological systems as observed in vivo studies. Thus it can sometimes be very challenging to extrapolate from the results of in vitro work back to the biology of the intact organism. In contrast to in vivo and in vitro studies, ex-vivo evaluation of functional foods offers an intermediate approach whereby living cells or tissues are directly subjected to therapeutic analysis outside the animals without involving prolonged cell culture. This approach forms the basis of analyzing possible in vivo effects of functional foods without actually performing the in vivo analysis. However, it also suffers the lack of a holistic scenario in terms of physiological effects of the food components.

Human trials

Although animal experimentation and in vitro approaches provide useful information concerning relationships between functional food components and their effects; properly performed randomized, controlled human intervention experiments yield the most robust evidence in support of food – health and disease/disease risk linkages. Indeed, well-designed clinical trials conducted using representative subject groups serve as a definitive benchmark globally for functional-food-based claims, such as structure-function claims that describe the role of a nutrient or food ingredient intended to maintain normal structure or function of the body in humans, and health claims that describe a relationship between a food, food component, or dietary supplement ingredient and reduction of risk of a disease or health-related condition. Clinical trials are the mandatory bridge between pre-clinical discovery of new medicinal products and their general uses. This means that clinical trials must take place before new research treatments can be made available to the public, whether for prescription, over-the-counter sale or for use in a clinic. Despite these, carrying out clinical trials has its own limitations:

- Results of trial may be different for different populations involving batch to batch variations.
- Randomized clinical trials usually only inspect one variable or very few variables, rarely reflecting the full picture of a complicated medical situation.
- Dosage variations in test compound used for full effects.
- Cost and ethical considerations of human trials
- Human trials generally take long time to provide substantial data for consideration
- Human trials are subject to both type I ("false positive") and type II ("false negative") statistical errors.

Conclusion

Dairy products are one of the potential candidates for functional foods due to several bioactive components. Research in these bioactive components in past decade has claimed health and nutritional benefits of milk based products. The commercialization of these products however, demands stringent scientific validation of purported claims using various techniques including human trials. Functional foods offer a unique advantage over drug development due to minimal toxicity or side effects. This should further make it possible to develop high throughput and effective means of validating dairy products as functional foods.
Suggested Readings


Science education is screaming for transformation. Research scholars and scientists need to be trained not only in their subjects, but also in terms of their soft skills and other life skills such as critical thinking, problem solving and communication proficiency. In Institutions of higher learning, the scientific writing is mostly perceived as short cuts, bullet points, technical terms, scientific equations and formulas. This is more of information processing rather than the knowledge sharing. There is no place for creativity or criticism. To move from information to knowledge, from experimental facts to rationale understanding of facts, we need language to express. Incomplete sentences only reflect inadequate learning and incomplete interpretation/ representation of facts. For creating new knowledge in science, we need to lay emphasis on the art of scientific writing. Effective communication depends a lot on our linguistic empowerment, which in turn, enhances our critical thinking and leads to cognitive empowerment.

Scientific writing 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 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.

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.

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.

**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?

**Some important language points and skills**

The ability to produce a clear, concise and professionally presented report is a skill that one is required to develop to be successful both at the research/academic institution and the workplace alike. A clear, concise and well-written report saves a lot of time of the users, be it researchers, students, teachers, managers or the clients. In other words, the value of accuracy and precision is not only important for researchers in scientific education and research but also for professionals in all sorts of work situations.

Successful communication depends upon the correct use of language and a good style of writing. One may learn the correct use of language, but has to cultivate a good style of writing. The former concerns grammar, usage, spelling, capitalization and punctuation, the latter concerns the organization of ideas through proper choice of words, arrangement of words into sentences, grouping of sentences into paragraphs, sections and chapters. The use of abbreviations, the approach to the reader, use of idioms, use of visual aids, the format and layout of the report are all aspects of style. Following are some of the some of the language skills that make scientific writing effective:

**Choice of words:** The primary objective of scientific writing is to transmit information briefly, clearly and efficiently. This can be achieved only through simple, direct and plain style. The first step towards a simple and clear style is to use simple language. One must choose a short word rather than a long word, a plain and familiar word rather than a fancy or unusual word and a concrete word rather than an abstract word.
Conciseness: Conciseness describes writing that is direct and to the point. Writing that is not concise is wordy. Wordy and indirect writing irritates the readers. In contrast, concise writing appeals to readers because it is direct. Hence, all efforts should be made to eliminate from the writing every word that does not contribute to the meaning or clarity of the message. Conciseness makes the writing clear and effective.

Discreet Use of Jargons: Jargon is specialized vocabulary of a particular group- words that an outsider unfamiliar with this field would not understand. Jargon encompasses all technical terms. Such terminology is useful and often necessary in technical communication restricted to people working on the same or similar subjects. Technical terms become jargon only when carelessly used for wider audience. Jargon is a special language of a particular field or profession. We can’t expect lawyers to say habeas corpus in English just because the rest of us don’t understand. The Jargon of any given field is often the most efficient means of communication within that field. It becomes offensive when handy English equivalents are available or people outside the field are expected to understand, what is said. In other words, using jargons unnecessarily is pretentious, showy, and artificial.

Avoid Colloquial Diction: Colloquial diction is a language that reads like spoken English. In some contexts, colloquial diction is perfectly appropriate. This is mostly used in fiction as conversational lines for the characters and is considered as a private style. In public style or technical reporting - colloquial diction is not desirable. e.g. The president was apparently unaware of his appointment with this real important guy in Moscow.

The Verb ‘Be’: The verb ‘be’ is often a cause of stylistic problems. Eight basic forms of verb ‘be’ are: am, are, is, was, were, be, being, been. Avoid verb ‘be’ followed by adjectives or nouns that can be turned into strong, economical verbs.

Appropriate Use of Coordination and Subordination: A common failing of technical writers is the expression of ideas of unequal importance in constructions that seem to give equal weight. Appropriate use of coordination and subordination should be made by carefully examining which ideas are important and which are minor, reworking them into simple, compound and complex sentences. Meaning can be grasped more quickly and more easily if subordinate ideas are indicated and put in subordinating constructions. A sentence should express the main thought in a principal clause. Less important thoughts should be expressed in subordinate clauses. Length of the sentence should be kept as short as far as possible by using not more than one or two subordinating conjunctions or relative pronouns in a sentence. There is a greater risk of grammatical error in longer sentences.

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.

References


Economic Importance of Traditional Indian Dairy Products

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Introduction

Milk and milk products constitute an increasingly large share of India’s food basket. Out of total food expenditure, milk and milk products account for about 19% and 15% share in urban and rural India, respectively (NSSO, 2011-12). The rising Gross Domestic Product (GDP) growth rate coupled with various government welfare programs is leading to increase of purchasing power resulting in shift from consumption of cereals to vegetables, milk and meat. This combined with increasing urbanization and increased emphasis on healthy eating has enhanced the demand for milk and milk products. To meet the increasing demand of milk and milk products, NDDB has designed National Dairy Plan, which envisages achieving an annual milk production of 200 million tonnes by 2021-22 from the level of 121.8 million tonnes in 2010-11 (NDP, 2012). The plan is aimed to give a major thrust towards increasing productivity of milch animals as well as to increase market share of the organized sector in collection of marketable surplus milk of the country for improving the milk handling and product quality.

From time immemorial, traditional Indian milk products have remained at an eminent position in Indian food culture. The range of traditional Indian dairy products represents the cultural diversity of India and its richness. In India, around 150 varieties of milk based traditional products are produced, many of them being region specific. Various traditional milk products are broadly classified into heat and acid coagulated products like chhana and paneer; heat dessicated products like kulfi, rabri, basundi and khoa; cultured product like dahi, mishti doi, shrikhand, lassi and chhach/matha; fat rich product like ghee and makkhan etc. Depending on regional preferences, these products have different variants. In addition to providing means of preservation of milk solids, production of traditional dairy products has immense economic importance. Manufacturing of traditional dairy products fetches additional income to farmers and provides considerable employment opportunities.

Share of milk and milk products in MPCE

India is undergoing transformations in its economy, changes in tastes and lifestyles, urbanization, and rising income levels. All of which are likely to have significant influences on food demand. With rapid increase in income and urbanization, food consumption in India has shown a pattern of change over the past three decades. From a diet primarily characterized by cereal staple foods; mainly rice and wheat, to one that includes a larger share of milk and dairy products, fruit, eggs, fish, meat, as well as processed foods. As expected, there has been a continuous shift of food expenditure in favour of high value foods.

The consumer expenditure survey, conducted by National Sample Survey Office (NSSO) aims at generating estimates of household monthly per capita expenditure (MPCE) and its distribution, separately for the rural and urban sectors of the country, for States and Union Territories, and for different socio-economic groups. In June 2013, NSSO in its 68th round survey has released the key
indicators of household consumer expenditure in India, generated from the data collected during July 2011 - June 2012 (NSSO, 2011-12). Consumption pattern trends during the last two decades indicate that since 1999-2000 in urban India and since 2011-12 in rural India non food expenditure has surpassed the food expenditure. The share of cereals within food expenditure has declined from 25.6 per cent in 1993-94 to 18.9 per cent in 2011-12 in urban areas and from 38.3 per cent in 1993-94 to 24.7 per cent in 2011-12 in rural areas. However, the share of milk and milk products within food expenditure has increased from 17.9 per cent in 1993-94 to 20.2 per cent in 2011-12 in urban areas, making milk and milk product the largest food expenditure category. In rural areas, the share of milk and milk products within food expenditure has also increased from 15.0 per cent in 1993-94 to 18.7 per cent in 2011-12.

Milk utilization pattern

According to Rabobank (2010) analysis, during 2009-10 of the total milk produced in India, about 41 per cent is retained by milk producing households for self consumption or sold to rural non-producers. Of the remaining 59 per cent of disposable milk, only 20 per cent is processed by the formal or organized sector, whereas a whopping 39 per cent is handled by informal sector comprising of traditional dudhias/halwais. The organized sector, comprising of co-operative, private and government dairies, disposes large portion of milk as packaged liquid milk and only surplus is converted into products. About 15 per cent of country’s total milk production is sold by organized dairy sector as liquid milk and about 5 per cent as value added milk products. In informal sector, about 17 per cent of total milk production is sold as loose milk, whereas about 22 per cent is converted into various traditional dairy products. This milk utilization pattern clearly shows the importance of traditional dairy products sector in the country's economy.

Economics of traditional dairy products

For long time, traditional dairy products remained backbone of traditional milk marketing system in India. The value added to milk through processing, product manufacturing and marketing by Indian halwais is nearly two times the price paid to the milk producers, while the western dairy products manufactured by Indian organized sector add about 50 per cent value to milk (Gupta, 2007). However, Punjrath (1991) noted that in spite of the fact that the dairy industry has made rapid strides through Operation Flood programme, the methods of manufacture of the traditional products remained essentially unchanged except for a few isolated products as most of the developments in dairy sector in India were directed towards manufacture of western dairy products for which equipment and processes were readily available from industrially advanced countries. Lohar and Killedar (1997) studied the cost of production and marketing of khoa, basundi, kunda and pedha in Western Maharashtra and reported 3.9 per cent net profit on total cost of production for khoa, whereas the same was increased to 6.6, 13.2 and 19.6 per cent for basundi, kunda and pedha, respectively, after conversion to sweets. The operating margin of traditional dairy products is higher, mainly due to lower raw material cost. It was reported that the raw material costs as percentage of sales prices of dahi, rasagolla, gulabjamun, peda, paneer etc. are much lower than those of butter and milk powder (Aneja, et. al., 2002; FAO, 1990). Chakraborty, 1998 studied the cost of production of chhana based sweets in Kolkata and reported that in all the products variable cost account for more than 98 per cent and fixed cost to less than 2 per cent of the total cost of production. Sangu, 2004 studied to find out the extent of profit generation and break-even level of production of locally produced khoa, paneer and ghee in Western Uttar Pradesh. Study revealed that in the cost of the products, major share was of raw material, followed by fuel and labour cost. The actual production of all the study products was more than their break-even levels, indicating profit of the business.
manufacturing is also studied in Hooghly and North 24 Parganas, two adjoining districts of Kolkata, and observed that variable cost contributed about 93.7 per cent and in absence of mechanized technology fixed cost contributed only 1.6 per cent of cost of production of chhana (Dutta, 2011). Among different components of variable cost, milk cost contributed about 82 per cent and fuel cost contributed to about 7 per cent of total cost of production and marketing. On an average chhana manufacturers make a net profit of about 5 per cent on total cost of chhana. However, calculated total returns earned by a chhana manufacturer are an understatement of their real earnings. The chhana manufacturers earn profit by way of extracting cream from milk and in some cases they do not incur many costs like household labour, rent of own house premises etc., and thereby earn profit of as high as 30 per cent on total cost of chhana.

International Dairy Federation Workshop on Small Scale Dairy Processing and Indigenous Milk Products (IDF, 1997) reported the cost aspects of small scale processing of indigenous dairy products and suggested that energy, which constitutes the major cost element of small scale processing, should be reduced by using efficient technology. Goel (1998) studied the techno-economic feasibility of commercial production of misti doi and indicated that the additional investment on purchase of equipments for commercial production could be recovered within a year. Solanki et. al., 2002 reported that traditionally, for khoa preparation, wood, coal or wood and kerosene are used at the village level. To make the system more efficient it was observed that steam jacketed open pan and diesel fired stoves are also being adopted. However, they reported that all these methods of khoa manufacturing suffer from major drawbacks, such as, batch-to-batch variation in product quality, small-scale batch process unsuitable for commercial adoption, and energy inefficiency. In this study, they compared the cost of utility and labour of various conventional methods along with NDDB developed mechanized method of khoa manufacturing and concluded that the utility and labour cost of mechanized system of khoa manufacturing was considerably lower than conventional methods.

Market of traditional dairy products

The Indian Food Sector is estimated to be INR 10,360 billion in 2009-10 and is expected to grow at a compounded annual growth rate of 8.1% for the next 5 years (KPMG, 2010). Dairy is one of the key contributors to the Indian Food Industry as it enjoys a dual distinction of both the world’s top milk producer and the world’s largest milk consumer. At INR 2490 billion in 2009-10, it formed a quarter of the food sector. Dairy market is expected to grow at a compounded annual growth rate of around 8% over the next 5 years from the base of 2009-10. According to a research by Tata Strategic Management Group (Gupta and Bhattacharya, 2010), the share of value added products in the overall packaged dairy products market in India has increased from 27 per cent to 36 per cent between 2005 and 2009, while the share of milk has decreased from 73 to 64 per cent during this period. The dairy sector in India is fast transforming from a commoditized-low margin liquid milk business to a branded value-added play. The Indian urban consumer today is willing to pay a premium for products that provide convenience and save time.

Traditional food products are preferred all over the world and chosen more in the developing world. According to GfK Roper Reports Worldwide, around 68 percent of global consumers and 71 percent of consumers of developing countries say they tend to stick with foods with which they are familiar (Tetra Pak, 2009). This phenomenon is evident from estimated growth in production of major traditional dairy products by cooperative sector dairies in India (Dutta and Bakshi, 2011). During the period of 1999-2000 to 2007-2008, production of dahi, gulabjamun, paneer, shrikhand, lassi and peda by cooperative dairies in India has witnessed 29, 28, 22, 18, 13 and 11 per cent compound annual growth rate respectively. According to IMRB Wallet Monitor Study, the average Indian household spent Rs. 35 per month on packaged dahi in 2010, up from Rs. 19 in 2007 (Business
World, 2012). Products like curd and lassi, which have traditionally been made at home, are increasingly being ‘brought to home’. Improving incomes and increasing hygiene consciousness are driving conversion from unpackaged to packaged products across food categories. Higher awareness towards health & hygiene, food safety and adulteration concerns are resulting in consumers converting from unorganized sources in traditionally unpackaged segments like paneer, curd, chaas and lassi to packaged/branded sources.

With increasing globalization, cross regional migration and greater cross cultural exposure to different food habits, the Indian consumer has started experimenting with various dairy products. This is increasingly leading to palate diversification and regional blending of tastes. Products like paneer and lassi are increasingly finding their way into South Indian homes. While the other zones witnessed a healthy 30-40 per cent increase in spends on paneer, the south zone where paneer has traditionally had little or no significance, witnessed a whopping 107 per cent increase in spends (Business World, 2012).

Export potential

India has been self sufficient in milk production and consumption. The exports of milk and milk products from India form less than 1 per cent of the total international trade. Also, exports account for less than 1 per cent of the total milk produced in India. The export market used to be mostly concentrated in Asia and more to neighboring countries and Middle East primarily due to low cost of end products. Concentrated milk products (skimmed milk powder) form bulk of exports from India.

A niche global market has strongly emerged for ethnic Indian dairy products. Indigenous milk products like shrikhand, gulabjamun, rasagolla, paneer, peda etc. have a market worth INR 5 billion in North America, Canada, Europe and Middle East (NAARM, 2003). Besides the NRIs, estimated to be 15 million, certain developed countries (mainly Canada, Australia, New Zealand and UK) have already taken major initiatives to cater to this market. Indications are that the market is fast growing with considerable future potential. There is an opportunity to take advantage of this niche market by developing dairy products of Indian ethnic origin meeting the quality and standards required for the global market.

Conclusion

The traditional dairy products and a great assortment of sweets are integral part of Indian heritage. Of great social, cultural and economic importance, these products have been developed over a long period of culinary skills and carry with them, the age-old wisdom and experience. In addition to preservation of the precious milk solids for longer duration, manufacture of traditional dairy products adds value to milk and also provides considerable employment opportunity.

Traditional dairy products offer considerable opportunities for the organized dairy industry of the country. Realization has set in, albeit late, that the traditional dairy foods have economic potential also for the organized industry. As a major dairying activity of the country, it is directly related to the livelihood of the milk producing farmers. Globalization of the dairy trade has further accentuated the need for directing scientific pursuits towards the recognition and promotion of the cultural significance attached to these traditional products.

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Instrumental Measurement of Food Texture

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Food sustains us. Rheological properties make us able to bite, chew and swallow it, while appealing to our senses. These properties are referred to as the “texture” of the food. The International Standards Organization (ISO) defines food texture in their standard for vocabulary for sensory analysis as “all the rheological and structure (geometrical and surface) attributes of a food product perceptible by means of mechanical, tactile, and, where appropriate, visual and auditory receptors” which further shows that rheology is an integral part of food texture. Texture measurement may be carried out by either Sensory analysis or Instrumental analysis. Sensory methods permit more complex attributes to be evaluated by the trained panellists and thus cost more to administer. Instrumental methods, on the other hand, are reliable within their limitations and are more economical to use.

Rheology is the science of flow and deformation of matter and describes the interrelation between force, deformation and time. Much of useful behavioural and predictive information for various products can be obtained only by rheological measurement. It is one of the key measurements for quality check during production and process monitoring/control.

Relation between different methods of food texture measurement:

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<tr>
<th>Characteristics</th>
<th>Method</th>
<th>Object</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective</td>
<td>Sensory evaluation</td>
<td>Perceived texture</td>
<td>Psychophysics</td>
</tr>
<tr>
<td></td>
<td>Mastication measurement</td>
<td>Oral process</td>
<td>Oral Physiology</td>
</tr>
<tr>
<td>Subjective</td>
<td>Instrumental</td>
<td>Food texture</td>
<td>Physics/Chemistry</td>
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Foods being diverse and complex materials in nature exhibit a wide range of different rheological properties, e.g., solids, liquids, plastics and viscoelastic behaviour. Varieties of different instruments have been developed to characterize their rheological properties. Instruments vary according to the type of deformation they apply to the sample, the property measured, the cost, the ease of operation, etc. Researcher must follow standardized test procedures to compare data from different laboratories.
Rheological properties determined using these techniques can be compared with measurements made by other workers in the literature or in other laboratories. A trend in the research in field of dairy and food sector has changed a lot during last decades. The single rheological instrument can analyse the very wide varieties of foods with different geometries. Viscometer and Universal texture analyser are two major such instruments which, when combined, can cover whole spectrum of rheological analysis.

Rotational Viscometers:

1. Rotational tests
In the controlled shear stress (CSS) tests, the shear stress is set and the shear rate is measured while in case of controlled shear rate (CSR) tests, the shear rate is set and shear stress is measured. In any of the method, the viscosity $\eta$ is calculated from the values of shear stress at corresponding shear rate. It is possible to determine individual viscosity values or a complete viscosity curve. The viscosity curve describes the viscosity dependence of the sample in a defined shear load range.

(A) Cylinder measuring system, (B) Cone/plate measuring system (CP), (C) Plate/plate measuring system (PP)

2. Oscillation tests:
In an oscillatory rheometer the moving part of the measuring system oscillates, creating vibrations which are transferred to the sample. This provides information on the viscoelastic behaviour of the substance. The oscillation is usually carried out at very low deformations to stay in the elastic range. In contrast to rotational tests, oscillatory tests have the advantage that the samples are not destroyed during testing. An oscillatory test simultaneously determines both the viscous behaviour, e.g. described by the loss modulus $G''$, and the elastic behaviour, e.g. described by the storage modulus $G'$. A variety of measuring systems allow both liquids and solids to be measured. This makes it possible to use one single instrument to investigate substances ranging from fluids such as water to cured sealing compounds, resins and even solid plastic.

(D) movement in rotational viscometry and (E) movement in oscillation viscometry
A. For **amplitude sweep test** (AST), varying amplitude at constant frequency and temperature is set. This test provides information on the limit of the linear viscoelastic range and the structural character of the sample. The following characters are defined: Gel or paste character with $G' > G''$ and liquid character with $G'' > G'$. The structural strength of a substance is expressed by the $G'$ value.

B. The **frequency sweep test** (FST) is carried out by varying the frequency at constant amplitude and temperature. Measurements at high frequencies represent the short-term behaviour of a sample. The long-term behaviour (storage stability) is described by measurement data at low frequencies.

C. The **temperature cycle test** (TCT) is carried out by varying temperature at constant amplitude and frequency. Temperature tests can be used to determine the glass transition temperature and melting temperature.

Apart from Rotational viscometry, Tube type viscometers can also be used which are very inexpensive as compared to rotational viscometers. The major disadvantage while using tube type is that the results lack precision and reproducibility. Latest instruments are based on the rotational viscometry.

**Texture Analysers:**

The principle of a texture measurement system is to deform the sample in predefined controlled conditions and simultaneous measurement its response. The graphical presentation of force response of the sample against the time/distance is shown on the digital display moving up or down depending on mode of compression using a probe or tension using grips. Forces created during these movements are manipulated to recreate conditions that foods are exposed to when we eat them or process them. These forces are a function of the properties of the sample and the parameters of the test method.

In general, texture analyser from various suppliers can be available with following accessories which are used for instrumental measurement of texture of various solids and semi-solid foods.

- Ball Probes
- Cone Probes
- Cylinder Probes
- Compression Plate
- Gel Bloom Kit
- Dough P
- Kramer Type Shear Cell
- Three Point Bend Food Jig
- Back Extrusion Cell
- Ottawa Forward Extrusion Cell
- Warner Bratzler Shear Blade Set
- Spaghetti/Noodle Test Fixture
- Puncture Probe Set
- Knife Edge Probe Set
- Gluten Dough Extensibility Jig
- Confectionery Accessories Kit
- Kramer Type Shear Cell
- Butter Cutting Jig
Parameters to be measured during Texture profile analysis of foods:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensorial definition</th>
<th>Instrumental definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness (H)</td>
<td>Force required to compress a food between molars. Defined as force necessary to attain given deformation</td>
<td>Peak force of the first compression cycle</td>
</tr>
<tr>
<td>Springiness (S)</td>
<td>Rate at which a deformed material goes back to its original condition after deforming force is removed</td>
<td>Height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite</td>
</tr>
<tr>
<td>Adhesiveness (A)</td>
<td>The work necessary to overcome the attractive forces between the surface of the food and the surface of other materials with which the food comes into contact (e.g. tongue, teeth, palate). Work required to pull food away from a surface</td>
<td>The negative area for the first bite, representing the work necessary to pull compressing probe away from sample</td>
</tr>
<tr>
<td>Cohesiveness (Co)</td>
<td>The strength of internal bonds making up the body of the product (greater the value the greater the cohesiveness)</td>
<td>The ratio of positive force during the second to that of the first compression cycle (downward strokes only)</td>
</tr>
<tr>
<td>Brittleness (B)</td>
<td>Force at which a material fractures. Related to the primary parameters of hardness and cohesiveness, where brittle materials have low cohesiveness. Not all foods fracture and thus value may relate to hardness if only single peak is present. Brittle foods are never adhesive.</td>
<td>The first significant break in the first compression cycle</td>
</tr>
<tr>
<td>Gumminess (G)</td>
<td>Energy required to disintegrate a SEMI-SOLID food product to a state ready for swallowing. Related to foods with low hardness levels</td>
<td>Calculated parameter: Product of Hardness x Cohesiveness</td>
</tr>
<tr>
<td>Chewiness (Ch)</td>
<td>Energy required to chew a SOLID food product to a state where it is ready for swallowing. attribute is difficult to quantify precisely due to complexities of mastication (shear, compression, tearing and penetration)</td>
<td>Calculated Parameter: Product of Gumminess x Springiness (or Hardness x Cohesiveness x Springiness)</td>
</tr>
</tbody>
</table>
Various rheological instruments being used for measurement of related rheological parameters:

<table>
<thead>
<tr>
<th>Product</th>
<th>Instrument to be used</th>
<th>Name of the test</th>
<th>Parameters to be evaluated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate liquor</td>
<td>Rotational viscometer</td>
<td>Viscosity curve</td>
<td>Viscosity, Yield stress</td>
</tr>
<tr>
<td>Chocolate (brick)</td>
<td>Texture analyser</td>
<td>Single bite compression, Warner-Bratzler cutting test</td>
<td>Hardness, Stickiness</td>
</tr>
<tr>
<td>Bread dough, cake batter and other Gluten based dough</td>
<td>Texture analyser</td>
<td>Constant strain rate extension test</td>
<td>Elongation properties</td>
</tr>
<tr>
<td></td>
<td>Rotational viscometer (PP)</td>
<td>Viscosity curve</td>
<td>Apparent Viscosity</td>
</tr>
<tr>
<td></td>
<td>Oscillation Viscometer</td>
<td>FST</td>
<td>Viscoelastic behaviour</td>
</tr>
<tr>
<td>Cheeses, Paneer, Starch based dairy desserts and Puddings</td>
<td>Texture analyser</td>
<td>Texture profile analysis</td>
<td>H, S, Ad, Co, B, G, Ch.</td>
</tr>
<tr>
<td></td>
<td>Oscillation Viscometer</td>
<td>Cone-penetration</td>
<td>Hardness</td>
</tr>
<tr>
<td>Set Yoghurt/Dahi and related products</td>
<td>Rotational viscometer (PP)</td>
<td>Viscosity curve</td>
<td>Apparent viscosity</td>
</tr>
<tr>
<td></td>
<td>Oscillation Viscometer (PP)</td>
<td>FST</td>
<td>Viscoelastic behaviour</td>
</tr>
<tr>
<td></td>
<td>Texture analyser</td>
<td>Single bite compression, Back extrusion</td>
<td>Hardness, Adhesiveness, Work of shear, Work of adhesion</td>
</tr>
<tr>
<td>Ice cream and frozen desserts</td>
<td>Oscillation Viscometer (CP)</td>
<td>AST, FWT, TCT</td>
<td>Viscoelastic behaviour</td>
</tr>
<tr>
<td></td>
<td>Texture analyser</td>
<td>Penetration test</td>
<td>Hardness</td>
</tr>
<tr>
<td>Plastic fat</td>
<td>Oscillation viscometer</td>
<td>AST, FST, TCT</td>
<td>Plastic behaviour, yield point</td>
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<tr>
<td></td>
<td>Texture analyser</td>
<td>Penetration test</td>
<td>Hardness</td>
</tr>
<tr>
<td>Edible vegetable oils</td>
<td>Rotational viscometer (PP)</td>
<td>Viscosity curve</td>
<td>Apparent viscosity</td>
</tr>
<tr>
<td>SCM, Cream, Table spreads, other food emulsions</td>
<td>Oscillation viscometer (CP)</td>
<td>TCT</td>
<td>Viscoelastic behaviour</td>
</tr>
<tr>
<td></td>
<td>Rotational viscometer (CP)</td>
<td>Viscosity curve</td>
<td>Apparent viscosity</td>
</tr>
<tr>
<td>Butter</td>
<td>Texture analyser</td>
<td>Wire cutting</td>
<td>Cutting strength</td>
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<tr>
<td>Instrument</td>
<td>Application</td>
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<tr>
<td>Adams Consistometer</td>
<td>Consistency of semi-solid fluid fruit purees</td>
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<tr>
<td>Armour Tenderometer</td>
<td>Beef tenderness</td>
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<tr>
<td>Ballauf Pressure Tester</td>
<td>Puncture testing of fruits and vegetables</td>
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<tr>
<td>BBIRA Biscuit Texture meter</td>
<td>Hardness of cookies and crackers</td>
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<tr>
<td>Bloom Gelometer</td>
<td>Puncture test of gelatins and jellies</td>
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<td>Extensigraph</td>
<td>Behaviour of wheat dough</td>
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<tr>
<td>Farinograph</td>
<td>Baking quality of wheat flour</td>
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<tr>
<td>Pea Tenderometer</td>
<td>Quality and maturity of peas</td>
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<tr>
<td>Texture analyser (Instron)</td>
<td>Texture of many foods</td>
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<td>Haugh meter</td>
<td>Egg quality</td>
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<td>Kramer shear press</td>
<td>Tenderness of peas and other particulate foods</td>
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<tr>
<td>Marine Colloids Gel Tester</td>
<td>Puncture test for marine extract gels</td>
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<td>Mixograph</td>
<td>Baking quality of wheat flour</td>
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<tr>
<td>Ottawa Texture Measuring System</td>
<td>Texture of many foods</td>
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<tr>
<td>Penetrometer</td>
<td>Firmness of butter and margarine</td>
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<tr>
<td>Ridgelimiter</td>
<td>Stiffness of pectin and fruit jellies</td>
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<tr>
<td>Succulometer</td>
<td>Maturity and quality of fresh sweet corn</td>
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<tr>
<td>SURDD Hardness Tester</td>
<td>Hardness of fats and waxes</td>
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<tr>
<td>Torry Brown Homogenizer</td>
<td>Toughness of fish</td>
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<tr>
<td>USDA Consistometer</td>
<td>Consistency of semi-solid fruit purees</td>
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<tr>
<td>Warner-Bratzler Shear</td>
<td>Toughness of meat</td>
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</tbody>
</table>

Khoa, Peda, Burfi, Kalakand, Chhana, Gulabjamun, Rasogolla, Sandesh and similar kind of TIDPs

Texture analyser
Texture profile analysis
H, S, Ad, Co, B, G, Ch.
References


