Advanced Course in Faculty Training

on

TECHNOLOGICAL DEVELOPMENTS
IN
CHEESE AND FERMENTED DAIRY PRODUCTS

JULY 5 - 25, 2011

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 (HARYANA)
LECTURE COMPENDIUM

OF

SILVER JUBILEE
NATIONAL TRAINING PROGRAMME
ON

Technological Developments
in
Cheese and Fermented Dairy Foods

July 5 – 25, 2011

CENTRE OF ADVANCED FACULTY TRAINING
(Dairy Processing)

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

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

Course Director

Dr. S.K. Kanawjia
Principal Scientist, Dairy Technology Division

Course Coordinators

Dr. Kaushik Khamrui
Sr. Scientist, Dairy Technology Division

Mr. Yogesh Khetra
Scientist, Dairy Technology Division

Dr. P. Narender Raju
Scientist, Dairy Technology Division

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

Dr. S. K. Kanawjia

Mr. Yogesh Khetra

Mr. Alok Chatterjee
Research Scholar, Dairy Technology Division

Cover Page Designed By

Mr. Alok Chatterjee

Mr. Yogesh Khetra

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ACKNOWLEDGEMENT

The Division of Dairy Technology of the National Dairy Research Institute received the status of Centre of Advanced Studies (CAS) in the year 1995-96 by the Indian Council of Agricultural Research (ICAR) in recognition of its significant scientific contributions, and its treasure of highly trained and qualified faculty as well as modern dairy processing facilities and laboratories. The Centre has recently been rechristened as “Centre of Advanced Faculty Training” (CAFT) by the ICAR. It has been striving to improve and upgrade the teaching, research and training capabilities of the Division as well as to develop competence of the faculty members of the State Agriculture Universities by disseminating recent advances in the area of Dairy Processing. In the past 24 training programmes in different areas of dairy processing have been successfully organized by CAFT. This is the 25th SILVER JUBILEE COURSE on "Technological Advances in Cheese and Fermented Dairy Foods" being conducted during July 5-25, 2011. As for the past course, this course also will be highly useful for the participating researchers and academicians in further developing their concepts in the area of fermented dairy foods.

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

I express my 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. His key role in successful implementation of the CAFT / DP programme is gratefully acknowledged. The continuing interest of Dr. S. L. Goswami, Joint Director (Research) and Dr. G. R Patil, Joint Director (Academics), NDRI, Karnal, in the CAS programme of the Division is truthfully appreciated.

I sincerely acknowledge the contribution of Dr. S. K. Kanawjia, Principal Scientist, Dairy Technology Division and the Course Director for meticulously planning and executing this course and also for timely publication of the course compendium. His unstinted efforts in working out all the details of this programme are indeed praiseworthy.

I express my thankfulness to the Guest Speakers, Dr. Shivasraya Singh, Ex-Joint Director (A), Dr. G. S. Rajorhia, Retd. Principal Scientist (DT), NDRI, Dr. B. D. Tiwari, Retd. Principal Scientist (DT), NDRI, Dr. A. J. Pandya, Professor and Head, Dept. of Dairy Processing & Operations, SMC College of Dairy Science, AAU, Anand and Dr. Satish Kulkarni, Head, NDRI-SRS, Bangalore.

I thankfully acknowledge the contribution of my colleagues Dr. D. K. Thompkinson, Dr. R. R. B. Singh, Dr. (Ms) Latha Sabikhi, Dr. A. K. Singh, Dr. Kaushik Khamrui, Mr. Prateek Sharma, Mr. Yogesh Khetra, Dr. P. N. Raju, Mr. G. S. Meena, Mr. Sathish and Mr. Devraja H.C. for their valuable suggestions and logistics support.

I also thank all the staff of Dairy Technology Division including Mr. Ram Swarup, Mr. S.K. Kharb, Ms. Prem Mehta, Mr. Hakim Singh and Ms. Kusum for their contribution in day-to-day affairs of the CAFT programme. I thank Mr. Yogesh Khetra (Scientist) and Mr. Alok Chatterjee, (Research Scholar) of the Division for providing valuable inputs in designing the cover page of the compendium and also for their valuable assistance in formatting the manuscripts of the lectures for the compendium.

Date: July 2, 2011.

(A. A. Patel)
# Programme

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<tr>
<th>Date</th>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>05.07.2011 (Tuesday)</td>
<td>09:30 am - 10:00 am</td>
<td>Registration</td>
<td>Mr. Yogesh Khetra</td>
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<td>10:15 am - 11:30 am</td>
<td>Inauguration</td>
<td>Venue: Mini Auditorium</td>
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<td>11:30 am - 01:00 pm</td>
<td>Visit to ATIC</td>
<td>Dr. D. S. Sohi</td>
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<td>01:00 pm - 02:00 pm</td>
<td>Lunch Break</td>
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<td>02:00 pm - 03:00 pm</td>
<td>Production and marketing of cheese-A global perspective</td>
<td>Dr. S. Singh</td>
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<td>03:00 pm - 03:15 pm</td>
<td>Tea Break</td>
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<td>03:15 pm - 04:30 pm</td>
<td>Visit to National Library of Dairying</td>
<td>Dr. B. R. Yadav</td>
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<td>04:30 pm - 05:00 pm</td>
<td>Introduction to CAFT (DP) Course on TDCFDF</td>
<td>Dr. S. K. Kanaujia</td>
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<td>06.07.2011 (Wednesday)</td>
<td>09:30 am - 11:15 am</td>
<td>Dairy operations in Model Dairy Plant</td>
<td>Mr. G. C. Mutreja</td>
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<td>11:15 am - 11:30 am</td>
<td>Tea Break</td>
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<td>11:30 am - 12:00 pm</td>
<td>Visit to Experimental Dairy</td>
<td>Mr. H. R. Gupta</td>
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<td>12:00 pm - 01:00 pm</td>
<td>Quality of milk in relation to cheese making</td>
<td>Dr. Raman Seth</td>
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<td>01:00 pm - 02:00 pm</td>
<td>Lunch Break</td>
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<td>02:15 pm - 03:15 pm</td>
<td>Probiotics as potential functional food ingredient in dairy foods</td>
<td>Dr. V. K. Batish</td>
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<td>03:15 pm - 03:30 pm</td>
<td>Tea Break</td>
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<td>03:30 pm - 04:30 pm</td>
<td>Probiotics as biotherapeutics for management of inflammatory metabolic disorders</td>
<td>Dr. (Ms) Sunita Grover</td>
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<td>07.07.2011 (Thursday)</td>
<td>09:30 am - 10:30 am</td>
<td>Nutritional aspects of cheese</td>
<td>Mr. Devraja H. C.</td>
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<td>10:30 am - 10:45 am</td>
<td>Tea Break</td>
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<td>10:45 am - 12:00 pm</td>
<td>Microbiological quality in relation to cheese making</td>
<td>Dr. Surajit Mandal</td>
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<td>12:00 pm - 01:00 pm</td>
<td>Milk coagulating enzymes in cheese manufacture</td>
<td>Dr. R. K. Malik</td>
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<td>01:00 pm - 02:00 pm</td>
<td>Lunch Break</td>
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<td>02:00 pm - 03:15 pm</td>
<td>Technology of fruit flavoured and fiber fortified fermented dairy products</td>
<td>Dr. P. Narender Raju</td>
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<td>03:15 pm – 03:30 pm</td>
<td>Tea break</td>
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<td>03:30 pm - 04:30 pm</td>
<td>Recent advances in symbiotic herbal (<em>Tulsi</em>) yoghurt – Food safety and shelf life enhancement</td>
<td>Dr. Chand Ram Grover</td>
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<td><strong>08.07.2011 (Friday)</strong></td>
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<tr>
<td>09:30 am – 10:30 pm</td>
<td>Milk-soy based fermented products</td>
<td>Dr. Shilpa Vij</td>
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<td>10:50 am - 11:10 am</td>
<td><strong>Tea Break</strong></td>
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<tr>
<td>11:10 am - 12:30 pm</td>
<td>Food safety as applied to fermented foods products</td>
<td>Dr. G. S. Rajorhia</td>
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<td>01:00 pm - 02:00 pm</td>
<td><strong>Lunch Break</strong></td>
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<tr>
<td>02:00 pm - 05:00 pm</td>
<td>Manufacture of Mozzarella cheese (Practical)</td>
<td>Dr. D. K. Thompkinson &amp; Mr. Ram Swarup</td>
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<td><strong>09.07.2011 (Saturday)</strong></td>
<td>To study dairy plant operations</td>
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<td><strong>10.07.2011 (Sunday)</strong></td>
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<td><strong>11.07.2011 (Monday)</strong></td>
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<td>09:30 am - 11:15 am</td>
<td>Developments in Dutch varieties of cheeses : Manufacture of Gouda cheese (Practical) (Continued)</td>
<td>Dr. (Ms) Latha Sabikhi &amp; Mr. S. K. Kharb</td>
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<td>11:15 am - 11:30 am</td>
<td><strong>Tea Break</strong></td>
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<td>11:30 am - 01:00 pm</td>
<td>Developments in Dutch varieties of cheeses : Manufacture of Gouda cheese (Practical) (Continued)</td>
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<td><strong>Lunch Break</strong></td>
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<td>02:15 pm - 03:15 pm</td>
<td>Developments in Dutch varieties of cheeses : Manufacture of Gouda cheese (Practical) (Continued)</td>
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<td>03:30 pm - 04:30 pm</td>
<td>Developments in Dutch varieties of cheeses : Manufacture of Gouda cheese (Practical) (Continued)</td>
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<td><strong>12.07.2011 (Tuesday)</strong></td>
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<td>09:30 am - 10:30 am</td>
<td>Fibre enriched functional cheese like products</td>
<td>Dr. R. R. B. Singh</td>
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<td>10:30 am - 10:45 am</td>
<td><strong>Tea Break</strong></td>
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<tr>
<td>11:00 am - 12:00 pm</td>
<td>Technology for manufacture of whey cheeses</td>
<td>Dr. S. K. Kanawjia</td>
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<td>12:00 pm - 01:00 pm</td>
<td>Analytical techniques for characterization of flavouring compounds in cheese</td>
<td>Dr. Rajesh Kumar</td>
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<td>01:00 pm - 02:00 pm</td>
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<td>02:15 pm - 03:15 pm</td>
<td>Technology of buffalo milk cheese</td>
<td>Dr. S. Singh</td>
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<td>03:15 pm - 03:30 pm</td>
<td><strong>Tea Break</strong></td>
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<td>03:30 pm - 04:30 pm</td>
<td>Developments in processed cheese and cheese spread</td>
<td>Dr. V. K. Gupta</td>
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<td><strong>13.07.2011 (Wednesday)</strong></td>
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<td>09:30 am - 11:00 am</td>
<td>Technology of Mozzarella cheese</td>
<td>Dr. S. K. Kanawjia</td>
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<td><strong>Tea Break</strong></td>
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<td>11:30 am - 12:30 pm</td>
<td>Imitation cheese products</td>
<td>Dr. V. K. Gupta</td>
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<td><strong>Lunch Break</strong></td>
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<td>02:15 pm - 03:15 pm</td>
<td>Technology and preservation of cottage cheese</td>
<td>Dr. B.D. Tiwari</td>
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<td>03:15 pm - 03:30 pm</td>
<td><strong>Tea Break</strong></td>
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<td>03:30 pm - 04:30 pm</td>
<td>Additives in cheese making</td>
<td>Dr. A. J. Pandya</td>
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<td><strong>14.07.2011 (Thursday)</strong></td>
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<td>09:30 am - 10:30 pm</td>
<td>Cheese-making equipment and mechanization</td>
<td>Dr. A. J. Pandya</td>
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<td>10:45 am - 11:00 am</td>
<td><strong>Tea Break</strong></td>
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<td>11:00 am - 01:00 pm</td>
<td>Renneting behavior of milk and curd forming characteristics (Practical)</td>
<td>Dr. B.D. Tiwari</td>
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<td>01:00 pm - 02:00 pm</td>
<td><strong>Lunch Break</strong></td>
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<td>02:15 pm – 03:15 pm</td>
<td>Genetic engineering of dairy starters and their applications in dairy products</td>
<td>Dr. Rameshwar Singh</td>
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<td><strong>Tea Break</strong></td>
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<td>03:30 pm - 04:30 pm</td>
<td>Fermenters and downstream processing</td>
<td>Er. I.K. Sawhney</td>
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<td>15.07.2011 (Friday)</td>
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<td>09:00 am - 11:15 am</td>
<td>Manufacture of processed cheese (Practical) (Continued)</td>
<td>Dr. D. K. Thompkinson &amp; Mr. Ram Swarup</td>
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<td>11:15 am - 11:30 am</td>
<td>Tea Break</td>
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<td>11:30 pm - 01:00 pm</td>
<td>Manufacture of processed cheese (Practical) (Continued)</td>
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<td>Lunch Break</td>
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<td>02:15 pm - 03:15 pm</td>
<td>Popular milk based fermented products of southern region</td>
<td>Dr. Satish Kulkarni</td>
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<td>03:15 pm - 03:30 pm</td>
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<td>03:30 pm - 04:30 pm</td>
<td>Biofunctional whey based functional beverages</td>
<td>Dr. (Ms) Shilpa Vij</td>
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<td>16.07.2011 (Saturday)</td>
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<td>09:00 am - 11:15 am</td>
<td>Manufacture of milk-cereal based fermented product (Practical)</td>
<td>Dr. A.K. Singh &amp; Mr. G. S. Meena</td>
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<td>11:15 am - 11:30 am</td>
<td>Tea Break</td>
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<td>11:30 am - 01:00 pm</td>
<td>Manufacture of milk-cereal based fermented product (Practical) (Continued)</td>
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<td>Lunch Break</td>
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<td>02:15 pm - 03:15 pm</td>
<td>Microstructure of reduced fat cheeses</td>
<td>Dr.S. K. Tomar</td>
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<td>Tea Break</td>
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<tr>
<td>03:30 pm - 04:30 pm</td>
<td>Technological advances in whey based fermented beverages</td>
<td>Mr. Sathish M.H.</td>
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<td>17.07.2011 (Sunday)</td>
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<td>18.07.2011 (Monday)</td>
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<td>09:00 am - 11:15 am</td>
<td>Rheological properties of cheese (Practical)</td>
<td>Mr. Prateek Sharma &amp; Mr. G. S. Meena</td>
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<td>Tea Break</td>
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<td>11:30 am - 01:00 pm</td>
<td>Rheological properties of cheese (Practical) (Continued)</td>
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<td>Lunch Break</td>
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<td>02:15 pm - 03:15 pm</td>
<td>Cheese – A potential source for bioactive peptides</td>
<td>Dr. (Ms) Bimlesh Mann</td>
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<td>03:30 pm - 04:30 pm</td>
<td>Developments in fermented dairy analogues</td>
<td>Dr. A. A. Patel</td>
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<td>19.07.2011 (Tuesday)</td>
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<td>09:00 am - 11:15 am</td>
<td>Electron microscopy in textural studies of bio-processed foods (Practical)</td>
<td>Dr. S. K. Tomar &amp; Mr. Ram Swarup</td>
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<td>11:30 am - 01:00 pm</td>
<td>Electron Microscopy in Textural Studies of Bio-processed Foods (Practical) (Continued)</td>
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<td>02:00 pm - 03:15 pm</td>
<td>Developments in Quarg cheese technology</td>
<td>Dr. S. K. Kanawjia</td>
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<td>03:30 pm - 04:30 pm</td>
<td>Technological advances in the manufacture of Misti Dahi</td>
<td>Dr. P. Narender Raju</td>
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<td>20.07.2011 (Wednesday)</td>
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<td>09:00 am - 11:15 am</td>
<td>Preparation of lassi/fruit lassi (Practical)</td>
<td>Mr. Yogesh Khetra &amp; Mr. Devraja H.C.</td>
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<td>Preparation of lassi/fruit lassi (Practical) (Continued)</td>
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<td>Safety aspects of cheese and fermented milk</td>
<td>Dr. Naresh Kumar Goel</td>
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<td>03:30 pm - 05:00 pm</td>
<td>Technology of Raabdi – A traditional cereal based fermented milk beverage</td>
<td>Mr. Yogesh Khetra</td>
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<tr>
<td>21.07.2011 (Thursday)</td>
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<tr>
<td>09:30 am - 10:45 am</td>
<td>Technology for manufacture of Feta cheese</td>
<td>Dr. S. K. Kanawjia</td>
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<tr>
<td>Time</td>
<td>Session</td>
<td>Speaker</td>
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<tr>
<td>10:45am - 11:00 am</td>
<td>Tea Break</td>
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<tr>
<td>11:00 am - 11:50 am</td>
<td>Rheological properties of cheese</td>
<td>Mr. Prateek Sharma</td>
<td></td>
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<tr>
<td>12:00 pm - 12:50 pm</td>
<td>Milk-Cereal based fermented products</td>
<td>Dr. A. K. Singh</td>
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<tr>
<td>01:00 pm - 02:00 pm</td>
<td>Lunch Break</td>
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<tr>
<td>02:15 pm – 03:15 pm</td>
<td>Bio protective metabolites produced by lactic acid bacteria</td>
<td>Dr. R. K. Malik</td>
<td></td>
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<tr>
<td>03:15 pm – 03:30 pm</td>
<td>Tea Break</td>
<td></td>
<td></td>
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<tr>
<td>03:30 pm - 04:30 pm</td>
<td>Recent developments in packaging of cheese</td>
<td>Dr. G.K. Goyal</td>
<td></td>
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<tr>
<td>22.07.2011 (Friday)</td>
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<tr>
<td>09:30 am - 11:00 am</td>
<td>Preparion of cheese spreads (Practical)</td>
<td>Dr. D. K. Thompkinson</td>
<td></td>
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<tr>
<td>11:00 am - 11:15 am</td>
<td>Tea Break</td>
<td></td>
<td></td>
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<tr>
<td>11:15 am - 01:00 pm</td>
<td>Preparion of cheese spreads (Practical) (Continued)</td>
<td></td>
<td></td>
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<tr>
<td>01:00 pm - 02:00 pm</td>
<td>Lunch Break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>02:15 pm – 03:15 pm</td>
<td>Technology for manufacture of <em>Kradli</em></td>
<td>Dr. G. R. Patil</td>
<td></td>
</tr>
<tr>
<td>03:15 pm - 03:30 pm</td>
<td>Tea Break</td>
<td></td>
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</tr>
<tr>
<td>03:30 pm - 05:00 pm</td>
<td>Nutritional and therapeutic benefits of fermented foods</td>
<td>Dr. Surajit Mandal</td>
<td></td>
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<tr>
<td>23.07.2011 (Saturday)</td>
<td></td>
<td></td>
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<tr>
<td>09:30 am - 11:00 am</td>
<td>Manufacture of Quarg cheese (Practical)</td>
<td>Dr. S.K. Kanawjia &amp; Mr. Yogesh Khetra</td>
<td></td>
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<tr>
<td>11:30 am - 11:15 am</td>
<td>Tea Break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:15 am - 01:00 pm</td>
<td>Manufacture of Quarg cheese (Practical) (Continued)</td>
<td></td>
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<tr>
<td>01:00 pm - 02:00 pm</td>
<td>Lunch Break</td>
<td></td>
<td></td>
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<tr>
<td>02:15 pm – 03:15 pm</td>
<td>Preparation of Pizza (Practical)</td>
<td>Mr. Yogesh Khetra &amp; Mr. Ram Swarup</td>
<td></td>
</tr>
<tr>
<td>03:15 pm - 03:30 pm</td>
<td>Tea Break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>03:30 pm - 04:30 pm</td>
<td>Preparation of Pizza (Practical) (Continued)</td>
<td></td>
<td></td>
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<tr>
<td>24.07.2011 (Sunday)</td>
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<td></td>
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<tr>
<td>09:30 am - 10:30 am</td>
<td>Sensory evaluation of cheeses</td>
<td>Dr. K. Khamrui</td>
<td></td>
</tr>
<tr>
<td>10:30 am - 11:15 am</td>
<td>Essentials of Good Technical Writing</td>
<td>Dr. (Ms) Meena Malik</td>
<td></td>
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<tr>
<td>11:15 am - 12:15 pm</td>
<td>Design and maintenance of cheese cold store</td>
<td>Dr. A. K. Dodeja</td>
<td></td>
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<tr>
<td>12:15 pm - 12:45 pm</td>
<td>Interaction with Faculty</td>
<td>DT Faculty</td>
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<td>01:00 pm - 02:00 pm</td>
<td>Lunch Break</td>
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<tr>
<td>02:00 pm - 03:00 pm</td>
<td>Course Evaluation</td>
<td>Dr. S. K. Kanawjia</td>
<td></td>
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<tr>
<td>03:00 pm - 04:30 pm</td>
<td>Valedictory Function</td>
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<td>S.No.</td>
<td>Title</td>
<td>Author</td>
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<td>Dr. S. Singh</td>
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<td>Technology of buffalo milk cheese</td>
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<td>Dr. S. K. Kanawjia</td>
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<td>Dr. S. K. Kanawjia</td>
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<td>Genetic engineering of dairy starters and their applications in dairy products</td>
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<td>Food safety as applied to fermented food products</td>
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<td>Prof. I. K. Sawhney</td>
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<td>Cheese – A potential source for bioactive peptides</td>
<td>Dr. (Ms) Bimlesh Mann</td>
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<td>19</td>
<td>Bio protective metabolites produced by lactic acid bacteria</td>
<td>Dr. R. K. Malik</td>
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<td>Quality of milk in relation to cheese making</td>
<td>Dr. Raman Seth</td>
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<td>Mr. Yogesh Khetra</td>
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<td>Microbiological quality in relation to cheese making</td>
<td>Dr. Surajit Mandal</td>
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<td>Dr. Surajit Mandal</td>
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<td>Technological advances in the manufacture of <em>Misti Dahi</em></td>
<td>Dr. P. Narender Raju</td>
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<td>Analytical techniques for characterization of flavouring compounds in cheese</td>
<td>Dr. Rajesh Kumar</td>
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<td>Dr. G. K. Goyal</td>
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<td>Safety aspects of cheese and fermented milk</td>
<td>Dr. Naresh Kumar</td>
<td>227 – 236</td>
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<td>Biofunctional whey based functional beverages</td>
<td>Dr. (Ms) Shilpa Vij</td>
<td>237-241</td>
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<td>Dr. (Ms) Shilpa Vij</td>
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<td>Nutritional aspects of cheese</td>
<td>Mr. Devraja H. C.</td>
<td>247 – 253</td>
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<tr>
<td>34</td>
<td>Technological advances in whey based fermented beverages</td>
<td>Mr. Sathish M. H.</td>
<td>254 – 258</td>
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<tr>
<td>35</td>
<td>Recent advances in symbiotic herbal (<em>Tulsi</em>) yoghurt – Food safety and shelf life enhancement</td>
<td>Dr. Chand Ram Grover</td>
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<td>Dr. (Ms) Meena Malik</td>
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<td>Developments in processed cheese and cheese spread</td>
<td>Dr. V. K. Gupta</td>
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<td>Imitation cheese products</td>
<td>Dr. V. K. Gupta</td>
<td>280-289</td>
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<td>Milk coagulating enzymes in cheese manufacture</td>
<td>Dr. R. K. Malik</td>
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<td>40</td>
<td>Rheological properties of cheese</td>
<td>Mr. Prateek Sharma</td>
<td>296-306</td>
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</table>
Cheese is a milk product of ancient origin and portrays the ethos of civilization and its cultural evolution. Cheese is one of the oldest foods of mankind. It is commonly believed that cheese evolved in the Fertile Crescent between the rivers Tigris and Euphrates in Iraq some 8000 years ago. The so-called Agricultural Revolution occurred here with the domestication of plants and animals.

It seems that the cheese originated accidentally as a result of the activities of nomadic tribes. Since animal skin bags were a convenient way of storing liquids for nomadic people, these were used for storing surplus milk. Fermentation of the milk sugars in the warm climate prevailing would cause the milk to curdle in the bags. The swaying animals would have broken up the acid curd during journeys, to produce curds and whey. The whey provided a refreshing drink on hot journeys, while the curds, preserved by the acid of fermentation and a handful of salt, became a source of high protein food supplementing the meager meat supply.

This activity gave rise to the assumption that cheese was evolved from fermented milks. It is perhaps more probable that the crude fermentations progressed in two ways. In one direction towards the production of liquid fermented milks such as dahi, yoghurt, laban koumis and Kefir and in the other direction through the drainage of whey through a cloth or perforated bowls, to leave solid curds which when salted became cheese.

It was a prominent article of the Greek and Roman diet as much as 2500 years ago. It is referred to in the Old Testament several times.

Cheese making has been an ‘Art’ handed down from generation to generation, and during that time even for the fastidious palate of the gourmet. There has been insufficient knowledge to guide both milk producer and cheese maker away from those errors, which so frequently occur during natural fermentations.

Until the 18th century, cheese making was essentially a farmhouse industry, but towards the end of the century scientific findings began to provide guidelines, which were to have an impact on the process of making and ripening cheese. Thus cheese making became an ‘Art with Science’. Now the mechanization and automation has been taken to such a high level that tonnes and tonnes of cheese can be produced without a touch of hand.
Definition of Cheese: The word “cheese” is derived from the Old English “cесь” which in turn was derived from the Latin “caseus” which means correct or perfect thing. Cheese may be defined “as the curd of milk separated from the whey and pressed into a solid mass” - though satisfactory, but too limited and vague from technical standpoint. Therefore, a relatively more complete definition is as follows:

Cheese is the curd or substance formed by the coagulation of milk of certain mammals by rennet or similar enzymes in the presence of lactic acid produced by added or adventitious microorganisms, from which part of moisture has been removed by cutting, warming and pressing, which has been shaped in mould and then ripened (also unripened) by holding for sometime at suitable temperatures and humidities (Davis, 1965).

There has been steady increase in the consumption of cheese in most countries worldwide, the annual growth rate in cheese consumption being over 3% with an acceleration expected due to worldwide trend of adopting Western consumption habits with a high level of cheese in the diet. About 40% of total world milk production is converted into cheese. The major cheese production has centred in Western countries. In 2008, 17.2 million tonnes of cheese was produced in the world, of which the European Union and United States accounted for more than 50%. Significantly, New Zealand exported 110,000 tonnes (over 75% of the production) and is the world’s number two exporter. Both Australia and Switzerland ranking third and fourth, respectively, exported almost 45% of their total production. All these three countries along with EU accounted for 80% of the total world exports of almost one million tonnes in 1993.

The scenario of cheese production in India is quite bright because of the fact that cheese has all the beneficial attributes of an ideal dairy product and the emergence on new global economic reforms based on globalization and liberalization in the marketing arena that has unfastened the entry to the India dairy industry to penetrate the large international cheese market. The growth pattern of cheese production in India has been quite encouraging, being 800 tonnes in 1977 and 1000 tonnes in 1980. It increased to about 3000 tonnes per annum in 1987. In 1994, the production was estimated at 8000 tonnes, against the installed capacity of 9000 tonnes. Against this production, the total demand estimated is placed around 18000 tonnes which is projected to exceed 30000 tonnes by 2000 AD. The growth pattern of cheese production is shown in Table 1 and 2.

**Table 1. World milk utilization pattern (%)**

<table>
<thead>
<tr>
<th></th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Butter</td>
<td>34</td>
<td>36</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>Cheese</td>
<td>13</td>
<td>28</td>
<td>40</td>
<td>42</td>
</tr>
<tr>
<td>Preserved milk</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Liquid milk and others</td>
<td>49</td>
<td>31</td>
<td>25</td>
<td>24</td>
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</tbody>
</table>
Table 2. World production of major dairy products (million tonnes)

<table>
<thead>
<tr>
<th>Product</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
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</thead>
<tbody>
<tr>
<td>Butter</td>
<td>4.07</td>
<td>4.12</td>
<td>4.12</td>
<td>4.11</td>
<td>4.14</td>
</tr>
<tr>
<td>Cheese</td>
<td>14.1</td>
<td>14.5</td>
<td>14.9</td>
<td>15.2</td>
<td>15.5</td>
</tr>
<tr>
<td>SMP</td>
<td>2.83</td>
<td>2.97</td>
<td>3.03</td>
<td>2.90</td>
<td>3.10</td>
</tr>
<tr>
<td>WMP</td>
<td>2.72</td>
<td>2.97</td>
<td>3.02</td>
<td>3.16</td>
<td>3.25</td>
</tr>
<tr>
<td>Milk</td>
<td>562</td>
<td>569</td>
<td>577</td>
<td>584</td>
<td>593</td>
</tr>
</tbody>
</table>

Fig.1. World cheese production  
(Source: IDF Bulletin 432/2008)

Fig.2 World cheese consumption (Kg/year)  
(Source: IDF Bulletin 432/2008)

**Mode of Utilization:** cheeses are highly diversified. Names of cheeses number about 2000, although many have little differences. The manufacturing procedure and curing through centuries have resulted in the production of the cheese which ranges in flavour from extremely mild to very sharp and in texture from semi-solid
to almost stone hard. Thus cheeses differ to varying degrees in nutritive value, appearance, flavour, texture and cooking properties. Consequently cheese is capable of satisfying a diverse range of sensory and nutritional demands. The use of cheese is extended by secondary processing methods to create an array of cheese-based products. The major usage levels (per cent of total cheese consumed) is: Natural, 39%; Dry, 28%; Processed, 13%; and low-fat, 20%. The use of cheese as food ingredient accentuated the need for specific and consistent properties, which must be attained with correct flavour synergistic with the food (Olson and Bogenrief, 1995). The comparative usage level of cheese in different food products is shown in Table 3 (Patel, 2005). Cheese maker can provide a range of different flavours, texture and compositional properties to suit a variety of needs. It requires knowledge about its functionalities which can be effectively exploited for the benefits of consumers.

Table 3. Utilization of cheese in different food products

<table>
<thead>
<tr>
<th>Food Product</th>
<th>Usage (as % of total usage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pizza</td>
<td>26</td>
</tr>
<tr>
<td>Snack Foods</td>
<td>17</td>
</tr>
<tr>
<td>Soups/Sauces/Dressings</td>
<td>15</td>
</tr>
<tr>
<td>Frozen Entrees</td>
<td>14</td>
</tr>
<tr>
<td>Baked Goods</td>
<td>11</td>
</tr>
<tr>
<td>Appetizers</td>
<td>7</td>
</tr>
<tr>
<td>Pet Foods</td>
<td>6</td>
</tr>
<tr>
<td>Rice/Noodle mixes</td>
<td>3</td>
</tr>
<tr>
<td>Shelf Stable Entrees</td>
<td>1</td>
</tr>
</tbody>
</table>

The natural cheese can be eaten as such or put on bread, biscuits, etc as slices. At the turn of this century, developments in melting processes, involving natural cheese at various ages, have given birth to a line of process cheese products with controlled flavour, texture and extended shelf life. In addition, various shapes, sizes, configurations and sliced versions are created to provide varieties with novel applications. The consumer can use these products as ingredients in cooking of several dishes or as ready-to-eat snacks. These products are designed to be consumed as a spread or as slices in sandwiches and function as dip or toppings on snacks. Cheese crackers are quite popular in Western countries. Natural cheese can be dried to prolong its shelf life. Dried products can be used in bakery products, soups, sauces, snacks, pasta products, ready meals, biscuits, fillings, cheese substitutes/imitations, etc. Cheese ‘Pakoura’ is getting popular in our country.

The scenario of cheese production in India is quite bright because of the fact that cheese has all the beneficial attributes of an ideal dairy product and the emergence of new global economic reforms based on globalization and liberalization in the marketing arena that has unfastened the entry to the Indian dairy industry to penetrate the large international cheese market. The growth pattern of cheese
production in India has been quite encouraging, being 800 tonnes in 1977 and 1000 tonnes in 1980. It increased to about 3000 tonnes per annum in 1987. In 1994, the production was estimated at 8,000 tonnes, against the installed capacity of 9,000 tonnes. Against this production, the total demand estimated is placed around 18,000 tonnes which is projected to exceed 30,000 tonnes by 2000 AD.

Cheese consumption opportunities exist around all meals – breakfast, lunch, dinner and in between meal snacks. We can, therefore, assume consumption of about 20-25 grams cheese per head twice a week in one form or another. Assuming the Indian population at 100 crores, this leads to an estimated potential annual demand of about 50,000 tonnes. This is the domestic potential in cheese demand. In this projection, the production and consumption of paneer, chhana and shrikhand is not included, though technically they are also classified as cheese.

Consumption of cheese is increasing very fast in our country. Process cheese spread and slices have proven to be an ideal bread-mate. There is a very high growth rate in consumption of bread. Butter the traditional spread for bread is now avoided by people due to its high fat content which is implicated with obesity and cardiovascular diseases. The other conventional spreads like jam and jelly are considered as junk food. The introduction of pizza in India has added new fillip for enhanced consumption of Mozzarella cheese.

**Historical development of cheese manufacture in India:** Before independence, demand for cheese was very limited, mainly from the expatriates and some Indian communities and individuals who were exposed to western cuisine. This demand was mostly met from imports and few cottage scale cheese plants around hill stations, where expatriates used to go for summer vacations or had settled down for good. The growth in demand for cheese continued to be small after independence. To meet this demand, some cheese manufacturing plants came up, mostly in private small scale sector and the cooperative sector.

The plants started on a typical farm house pattern, with very simple and minimal cheese making equipment to keep the capital costs low. The cheese making technology was traditional farm house type with virtually no mechanization and/or automation. The small production capacities did not justify heavy investments in technology. Lack of cold-chain in the country also precluded marketing of natural cheeses. Only persons staying close to cheese manufacturing plants could enjoy the luxury of consuming natural cheeses. Most cheese was converted into process cheese for ease of marketing. Till about a decade back, the only major players in cheese market were Amul, Verka and Vijaya, all from the cooperative sector. These plants continued to expand their cheese manufacturing capacities slowly in tune with the demand growth for cheese.

During this period, many small cheese manufacturing plants also came up in different parts of the country in the private sector. These plants generally meet the
captive local demand from hotels, restaurants, food service sectors and consumers around their area of operations. These plants were mainly instrumental in diversifying into some more cheese varieties beyond Cheddar.

Economic liberalization in India has also coincided with increasing acceptance of cheese as a food by the Indian consumer, thus accelerating the growth in demand for cheese. This has resulted in the existing cheese manufacturing plants expanding their production capacities and upgrading their cheese manufacturing and packaging technologies. Many new players like Dynamix, Dabon, Vadilal, vintage, etc have entered the cheese manufacturing arena in recent years.

**Product mix for cheese plants:** Cheese being an alien product to India, only very selective mild flavoured varieties of cheese may be accepted by the average Indian consumers. With India now emerging as the largest and most competitive milk producer in the world, export of cheese to the neighbouring countries and even the advanced dairy countries has become a distinct possibility to be exploited. The emerging cheese market in India can be broadly segmented into two distinct classes:

- **Varieties for bulk users and consumers:** Cheddar including processed cheese and cheese spreads and Mozzarella.
- **Varieties for the connoisseurs:** Gouda, Edam, Swiss, Parmesan, Cottage, etc

India is bestowed with unique opportunity of being the largest producer of buffalo milk. Buffalo milk is considered especially suitable for manufacturing certain varieties of cheeses, viz. Mozzarella, Feta, Domiati, Ras and Paneer. With the popularity of pizza and other Italian/continental baked dishes increasing by leaps and bounds, demand for good quality Mozzarella cheese for baking pizzas, etc at home is increasing all over the world. The Middle-east countries and South-east Asia are the largest importer of cheese. Indian dairy industry should capitalize on this positive aspect to capture export markets for cheese.

**Marketing of cheese:** Marketing approaches to cheese will be dictated by the market segments the cheese manufacturer has planned to focus on. Markets for cheese can be broadly divided into institutional markets and consumer markets.

**Institutional markets:** For bulk institutional buyers like the food chains, consistency in quality and competitive price are the most important considerations. Only cheese plants which can invest in mechanization and automation of cheese manufacturing facilities can foot the bill in the long run for meeting the quantitative and qualitative demands from such bulk consumers.

In fact such plants should simultaneously focus on the export markets to attain production volumes to justify the heavy investments required in capacity, mechanization and automation. If they can guarantee consistency in product quality and assurance about food safety to the international buyers, there is no reason why they can't succeed on the export front. There is no doubt that our price will become
increasingly competitive in the world as the subsidies on milk and milk products are gradually withdrawn by the developed countries under the WTO regime.

**Consumer markets:** On the consumer marketing front, advertising will play an important role in inducing more and more consumers to consume cheese. The focus of marketing would be on modern life styles, convenience of consuming cheese at home and outside the home and nutritional superiority of consuming cheese vis-a-vis other milk based and non-milk based protein rich foods.

Developing and sustaining strong brands for cheese will be the key to securing strong consumer markets. Brand building is again a long-term investment which only strong national or international players can indulge in. Against the old established brands like Amul, Verka and Vijaya, we now have new brands like Britannia and Dabon in the market. The brand war for cheese is going to become more intense in future as some more players are expected to join in.

**Global competition:** In India, most of the cheese consumed is of process type, primarily packaged in tins. It is a derivative of Cheddar cheese. It possesses a mild flavour, judged from the point of a cheese connoisseur.

So far the Indian dairy industry has marketed what they have produced. But, it is believed that future cheese market in India will be governed by competitive forces of free-market economy. This mode will require market orientation in which production is geared towards consumer preference and demand. Market orientation will gradually replace production orientation. This is especially true for cheese destined for global markets. On the export front, India will face competition from EU, Australia, Switzerland, New Zealand, etc. The total export sales in the world market in 1993 were 940,000 tonnes, accounting for 6% of global cheese production.

![Top Cheese Exporters (Whole Cow Milk only) - 2004](http://en.wikipedia.org/wiki/Cheese)

**Fig. 3. Major cheese exporters in 2004**

(Source: http://en.wikipedia.org/wiki/Cheese)
International cheese trade in 2007 continued to grow regardless of the higher prices, only in the first month of 2008 it has declined. By far the biggest exporter is the EU with growing volumes until 2007. New Zealand, in second position, reduced exports because of the reduced domestic production, whereas Australia increased exports slightly. The US exported significantly more and has just doubled the export volumes of the year 2000. After strong losses in 2006 the Ukraine exports recovered after the lift of the temporary ban by Russia. Switzerland increased both the exports and imports since the free trade agreement for cheese with the EU was established in the middle of 2007. Still the biggest cheese importer is Russia, followed by Japan. Both countries are producing more and more cheese but not enough to cover their domestic needs. There and in many other parts of the world, the consumer demand is partly focusing on varieties from abroad, which is one reason for the growth of the cheese trade. The other reason is the general upward trend of cheese consumption, which also applies to the use of cheese as ingredient in other foods in catering and food services. It is heartening that India has also started exporting cheese in a modest way. It exported cheese worth U.S. $ 568,000 in 2004. This does not include shrikhand and paneer which are also technically classified as cheese.

Competing in global market will demand application of principles of Good Manufacturing Practices (GMP), commonly practiced in the US and EU. Indian dairy industry must adhere to stringent physical and chemical specifications to win consumer confidence in the export market. There is a need to develop and maintain export quality standards with a view to establish credibility of Indian dairy products in the world market. We must, at least, ensure that the shelf life of our products is comparable to that of our competitors from EU, New Zealand and Australia. Otherwise, we just can not stand in the global market. Furthermore, attention must be focused on producing cheese free from pathogenic microorganisms, viz. Listeria, Enteropathogenic E. coli, Salmonella sp, etc.
Technology of buffalo milk cheeses

Most of the well known cheese varieties of the world are conventionally produced from cow milk. However, buffalo milk too has been utilized with considerable success for manufacture of certain varieties of cheeses. Cheese made from buffalo milk displays typical body and textural characteristics. More specifically, where chewing and stringing properties are especially desired as in case of Mozzarella cheese, buffalo milk is technologically preferable over cow milk. In Italy, fresh and Pasta Filata cheese, especially Mozzarella has been traditionally prepared from buffalo milk. In Balkan countries, several types of white brined and pickled cheeses are prepared from buffalo milk. Feta (Greece), Domiati (Egypt) and Queso Blanco (South and Central America), Paneer (India) are among the prominent cheeses mainly prepared from buffalo milk. In countries where buffalo milk predominates, several cheese varieties are now manufactured from buffalo milk, which were earlier prepared from cow milk. An emerging market for buffalo milk cheeses in Western Europe, particularly as speciality and India has given a new dimension to the buffalo milk industry.

The manufacture of cheese originated and flourished in countries with relatively cold climate where cows are the main milk animals. Consequently, methods of cheese manufacture were developed for cow milk and emphasis was given to those varieties for which cow milk happens to be most suitable. In contrast, in our country the major share of milk is from buffaloes. Buffalo milk is not considered suitable raw material for making certain ripened cheese varieties, viz. Cheddar, Gouda, Emmental, etc. Ripened varieties are characterized by their soft, mellow and velvety body and texture and rich pleasing flavour. The cheese made from buffalo milk results in flat flavour and hard, rubbery and dry body and texture. This is mainly because buffalo milk differs markedly from that of cow milk both quantitatively and qualitatively. The high buffering capacity of buffalo milk due to its higher calcium and casein content is the cause for slower development of acidity. Faster renneting time may be attributed to its higher colloidal calcium content (~160 mg/100 ml as compared to only 8 mg/100 ml cow milk. Lower retention of moisture in the curd may be the result of low hydration of its casein compared to cow milk casein. Hard, rubbery and dry body may be due to high curd tension which, in turn, is the result of higher content of casein with bigger size of the micelles, high content of calcium and magnesium more so in the colloidal state, larger proportion of solid fat with bigger
size of globules and low voluminosity and solution of its casein micelles compared to the same in cow milk

As a result, the most common variety, the Cheddar cheese made in India does not develop proper flavour and body and texture when it is made from buffalo milk. The major problem is considerably faster rate of renting and syneresis which result in lower retention of moisture in the finished product. This, in turn, affects adversely the three most important biochemical reactions, i.e. glycolysis, proteolysis and lipolysis which constitute the cornerstone of cheese flavour development. In order to overcome this problem attempt should be made to develop a manufacturing technology which would ensure greater retention of moisture and accelerated rate of ripening.

Development of appropriate technology for cheddar cheese manufacture

Systematic work to develop a method for manufacture of Cheddar cheese from buffalo milk was initiated at the National Dairy Research Institute, Karnal in 1962. Almost simultaneously Dr J. Czulak, an Australian scientist developed a method while working at AMUL, Anand. Later on a pre-salting method was developed at NDRI for manufacture of Cheddar cheese. All these methods suffered from one or the other problem and therefore could not be adapted by the dairy industry at commercial scale. Recently, a method was developed at NDRI which results in quite acceptable cheese from buffalo milk (Singh and Kanawjia, 2000). The salient features of the newly developed method are as follows:

- **Heat treatment:** Normally sub-pasteurization temperature (thermization) is preferred to heat cow milk for cheese manufacture. Thermization involves heating of milk at 65°C for 15 seconds. In contrast, in case of buffalo milk it was observed that heating milk up to 69°C/30 min was more conducive to develop better cheese flavour. This may be due to partial precipitation of colloidal calcium, changes in casein micelles and interaction of casein micelles with whey proteins.

- **Ripening of milk (acidification):** In case of cow milk about 1% active lactic culture is used for ripening of milk. A rise of 0.02% acidity in about 45-60 min is considered to be satisfactory for adding rennet. Since in case of buffalo milk acidity development is relatively slower due to its higher buffering capacity the lactic culture is added at the elevated level of about 2%.

- **Supplementation starter culture with starter adjuncts:** It was observed that supplementation of regular starter culture with Lactobacillus casei str. 300 or L. helveticus at the rate of 0.5% improved flavour development in buffalo milk cheese.

- **Ripening temperature of milk:** It was found that relatively lower temperature of ripening of milk (28°C) in case of buffalo milk was more conducive for acidity development as compared to the higher temperature (30°C) in case of cow milk.
• **Cooking temperature:** Relatively lower cooking temperature in case of buffalo milk cheese (37°C/40-45 min) is helpful in retention of greater amount of moisture as compared to cow milk cheese (39-40°C/ 1h).

• **Cheddaring:** During cheddaring piling and repiling of cheese blocks should be more frequent to ensure greater retention of moisture in case of buffalo cheese.

• **Pressure:** Lower pressure should be applied on cheese block in case of buffalo milk cheese as compared to cow milk cheese.

• **Application of starter culture adjuncts and enzyme preparation:** In order to accelerate the ripening of cheese further from buffalo milk application of starter adjuncts and exogenous enzyme preparations should be made.

**Buffalo milk: boon for vegetarian cheeses**

Buffalo milk proved to be a boon for the manufacture of vegetarian cheeses. In India considerable proportion of the population is vegetarian. Milk and milk products are considered most appropriate food for vegetarians. However, during the manufacture of cheese an enzyme called rennet is used for coagulation of milk. This enzyme plays further role during ripening of cheese for the development of desirable body, texture and flavour. This enzyme is extracted as a byproduct from the lining of calf-stomach during the slaughter of the animals. Therefore, use of this enzyme for the manufacture of cheese is frowned upon by the vegetarians. In order to protect the feelings of the vegetarians the import of calf rennet was banned by the Government of India in mid eighties. The use of microbial rennet has a tendency to result in weak body and bitter flavour. The flavour defects result because the microbial rennets are more proteolytic causing relatively high degree of proteolysis in cheese. This problem was overcome by introducing certain modifications in the standard method of cheese manufacture in the ripened varieties.

Buffalo milk is relatively more resistant to the biochemical changes like glycolysis, proteolysis and lipolysis. Therefore, use of microbial rennet is unable to bring too extensive changes in lactose, proteins and lipids of cheese made from buffalo milk. The net result is that the occurrence of flavour defects in cheese made from buffalo milk using microbial rennet is minimized. Thus buffalo milk proved to be a boon for making ripened cheese varieties when microbial rennets are used.

**Accelerated ripening of cheese**

Cheese ripening times vary from 4 weeks to 28 months depending on the variety of cheese (Table 1). It is during ripening that special characteristics can develop, e.g. soft and mellow body and rich flavour of Cheddar cheese, blue veins in Roquefort, the holes (eyes) in Emmental, the red smear on Limburger, or the white mould on Brie. During ripening, the conditions of temperature and humidity are controlled carefully to promote the development of the desirable microflora and the secretion
of the enzymes responsible for the biochemical changes taking place during ripening.

The ripening of cheese is a slow and expensive process and can not be completely controlled. The cost of cheese ripening is quite high; for example, ageing time for Cheddar cheese adds significantly to the product costs, ranging from 1.5-3.0 % per month. The development of an efficient way to reduce the ageing time would result in significant savings to the cheese industry. Moreover, the industry has to meet a year-round demand for cheese from a highly seasonal production. By using both the accelerated ripening and normal ripening conditions, it may be possible to alleviate shortage of mature cheese at different times caused by the seasonal nature of production. Since ripening changes are relatively slower in case of buffalo milk cheese, the product becomes more expensive. Therefore, there is a need to enhance the ripening process of cheese. Several approaches are followed for this purpose:

It was only in the early 1950s that the economics of cheese making led dairy researchers to look for an appropriate method to reduce the maturation time for cheese, without altering the characteristic flavour of the final product. Over the last 50 years, various methods have been described that attempt to achieve this goal.

<table>
<thead>
<tr>
<th>Cheese Variety</th>
<th>Ripening Times (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheddar-type cheese</td>
<td>6-12</td>
</tr>
<tr>
<td>Swiss cheese varieties</td>
<td>6-12</td>
</tr>
<tr>
<td>Provolone</td>
<td>10-12</td>
</tr>
<tr>
<td>Parmigiano</td>
<td>24-28</td>
</tr>
<tr>
<td>Blue cheese varieties</td>
<td>3-4</td>
</tr>
<tr>
<td>Ras</td>
<td>3-4</td>
</tr>
<tr>
<td>Gouda</td>
<td>1-2</td>
</tr>
</tbody>
</table>

Strategies used for the enhancement of cheese ripening

- Elevated ripening temperature
- Slurry systems
- Stimulation of starter culture
- Adjunct cultures
- Modified starters
- Addition of enzymes
- Microencapsulated agents
- Supplementation with goat milk.

**Elevated ripening temperature**: Cheese was traditionally ripened in caves temperature varying from 15-20°C. Today, ripening takes place in special rooms under controlled temperature and humidity, which vary according to the variety of the cheese. Camembert, Blue cheese and surface ripened cheese are kept at 10-15°C, Gouda and Edam are usually ripened at 10-14°C, while a relatively low temperature is used for Cheddar (6-8°C). Several attempts were made to accelerate
Cheddar cheese ripening by increasing the temperature to 13-16°C, which led to a 50% increase in the rate of flavour formation. There was significant improvement in flavour development in buffalo milk Cheddar cheese when it was ripened initially at 15°C for 3-6 weeks followed by transferring at 8°C for subsequent ripening.

Although increasing the ripening temperature may offer the industry a technologically a simple method by which to speed up flavour-forming reactions in a cheese system, attention should be paid to the quality of the milk and hygienic conditions used for cheese production to avoid flavour defects and the growth of pathogens.

**Slurry system:** Cheese slurries are prepared by adding water and salt to cheese. Other additives include trace elements and reduced glutathione, which promote microbial growth and flavour formation in order to obtain a paste containing about 40% solids and showing a strong characteristic flavour. The mixture is incubated at 30°C for 4-5 days with daily agitation (Singh and Kristoffersen, 1968).

Cheeses slurries are used in a wide range of products, e.g. process cheese formulations, snacks, crackers and imitation dairy products. They are also an ingredient in the production of enzyme-modified cheeses. The major disadvantage of this technology is the difficulty of controlling this process. Contamination due to high incubation temperature is also likely to occur.

**Stimulation of starter culture:** The growth of starter culture may be stimulated by the addition of enzymes or hydrolysed starter cells to cheese milk. Ripening of Emmental type cheese has been accelerated by using starters grown to high cell numbers in media supplemented with protein hydrolysates (Law, 1990) and metalloproteinase from *Micrococcus caseolyticus* (Vassal, 1982). Significant contribution of non-starter lactic acid bacteria in increasing the rate of casein degradation and flavour development received much attention. Augmentation of starter culture with *L. casei* had definite positive influence on the flavour, body and texture of buffalo milk Cheddar cheese (Rao, 1991). The flavour development and biochemical changes in buffalo milk Cheddar cheese were enhanced by supplementing starter cultures with *L. casei, L.helveticus* and also with addition of heat and cold-shocked starters (Kanawjia and Singh, 1991).

**Starter adjuncts:** The development of procedures leading to a reduction in the number of microorganisms in milk at farm level resulted in a dramatic decrease in the number of non-starter bacteria for their positive role in cheese flavour development. Bacterial cultures have therefore been selected for their enzyme potential and autolytic properties and are added to cheese milk to supplement the microflora and enhance cheese flavour and texture development. Two different approaches were followed:

- Viable cells were used, where emphasis is given to metabolites produced by the living cells during cheese ripening.
• Weakened (attenuated) cells are used, which increase the pool of bacterial enzymes in the cheese matrix without altering the rate of acid production during cheese making.

Different means for attenuating cells have been described. In heat shocking, a bacterial cell suspension is subjected to sublethal temperature. A critical point in heat attenuation is to define the correct temperature/time combination so that acidification is eliminated or delayed without significant loss of activity of potential ripening enzymes. The conditions used to heat shock several microorganisms are summarized in Table 2. The attenuated cells are added to cheese milk along with the primary starter culture. *Lactobacillus helveticus* is the bacterial species most often subjected to heat treatment. An increase in proteolysis and the level of amino acids in cheese, as well as a decrease in bitterness and an enhancement of cheese flavour, have resulted from the addition of heat-shocked cells to cheese.

In freeze shocking, whole cells that were washed and concentrated 10-fold are subjected to freezing overnight or longer (Table 2), followed by thawing at 40°C prior to addition to milk.

The addition of freeze-shocked cells to cheese significantly increases proteolysis and amino acid formation due to cell lysis and the release of intracellular peptidase into the cheese. Attenuation by freezing is easier to achieve than attenuation by heat. However, no studies in which both treatments were applied to the same starter strains have been reported; therefore, their respective impacts on cheese ripening are difficult to compare.

Spray-drying or freeze-drying has also been used to attenuate bacterial cells; however, the results are not conclusive because of the rather limited number of studies carried out. Mutants that are unable to ferment lactose also provide an effective means for increasing enzyme potential in cheese without interfering with the rate of acid production during cheese making. Reduced bitterness and accelerated flavour formation were observed in Gouda and Cheddar cheese made with lac- mutants. Another attenuation method involves the treatment of the bacterial cells with lysozyme, which does not seem to be effective because of the difference in the sensitivity of lactic acid bacteria to lysozyme. In addition, relatively high price of lysozyme presents another obstacle.

Addition of modified/attenuated starter culture is to increase the number of starter cells without causing detrimental effect on the acidification schedule during manufacture so that the cells contribute only to proteolysis and other changes during ripening. Modified starter cultures with attenuated acid producing abilities are added with normal starter cultures during cheese manufacture. Selection of starter strains with enhanced autolytic properties and increased peptidase activity would provide a more balanced flavour.
Petterson and Sjostrom (1975) used heat-shocked cultures to attain large number of a mixed-strain starter, containing *Lactococcus*, *Leuconostoc* or *L. helveticus* strains which were cultivated at a constant pH, followed by heating to 69°C/15 s. Flavour score increased with increasing number of heat-shocked cells reducing the ripening time by 50 per cent. Addition of heat-shocked lactobacilli was found to increase peptidolysis and produce good flavour in low-fat semi-hard cheese (Ardo and Larsson, 1989). Flavour acceleration could be significantly improved by augmentation of starter culture with freeze-shocked *L. helveticus* in buffalo Gouda cheese (Malhotra, 1991).

A critical advantages and disadvantages of viable versus nonviable cells led to the conclusion that a combination of both is required in order to obtain the balanced flavour bouquet that characterizes each type of cheese.

### Table 2. Conditions used to attenuate different lactic cultures

<table>
<thead>
<tr>
<th>Strain</th>
<th>Temperature for physical attenuation</th>
<th>Temperature (°C)</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus helveticus</td>
<td></td>
<td>67</td>
<td>15 s</td>
</tr>
<tr>
<td>L. delbrueckii subsp. bulgaricus</td>
<td></td>
<td>63</td>
<td>20 s</td>
</tr>
<tr>
<td>L. casei</td>
<td></td>
<td>67</td>
<td>22 s</td>
</tr>
<tr>
<td>L. plantarum</td>
<td></td>
<td>50</td>
<td>15 s</td>
</tr>
<tr>
<td>L. helveticus</td>
<td></td>
<td>-24</td>
<td>24 h</td>
</tr>
<tr>
<td>L. casei</td>
<td></td>
<td>-20</td>
<td>20 h</td>
</tr>
<tr>
<td>L. lactis subsp lactis</td>
<td></td>
<td>-20</td>
<td>20 h</td>
</tr>
</tbody>
</table>

(Source: M. El. Soda, 2003, encyclopaedia of Dairy Sciences, pp328.)

**Cell-free extracts:** Addition of cell-free extracts to accelerate ripening generally leads to the accumulation of small peptides and free amino acids without an increase in gross proteolysis (Ezzat, 1990). Cell-free extracts of *L. helveticus*, *L. bulgaricus* or *L. lactis* are reported to increase primary proteolysis by enhancing β-casein breakdown (El-Soda, *et al.*, 1982). Ripening period of Cheddar cheese could be reduced to 2 months by the synergistic action of Neutrase and CFE of *L. lactis subsp. lactis* or *Brevibacterium linens* (Hayashi, *et al.*, 1990).

**Addition of enzymes:** Cheese ripening is essentially an enzymatic process and hence it should be possible to accelerate ripening by augmenting the activity of key enzymes. Flavour formation in cheese results from the action of the different agents involved in the ripening process. A great deal of attention has, therefore, been given to the different groups of enzymes that could play a role during ripening. Proteinases, peptidases, lipases and esterases from various sources have been added individually or in combination to cheese milk or to the cheese curd to speed up the ripening process. Enzymes extracted from the cheese-related microorganisms have also been considered. Lipases are used commercially in cheese varieties known for their characteristic piquant flavour, e.g. Provolone,
Cacciocavallo, some blue-veined cheeses, Ras cheese and Feta made by ultrafiltration. Very little attention has been directed towards enzymes involved in the metabolism of carbohydrates and has been limited to β-galactosidase. Addition of β-Galactosidase (lactase) to cow and buffalo cheese milks has been reported to reduce manufacturing time, accelerate ripening and improve flavour (Kanawjia, 1987). Marschke et al. (1980) found that lactase from Kluyveromyces lactis available as Maxilact contained a protease which was responsible for the increased level of peptides and free amino acids in cheese. However, addition of single enzyme which accelerates one particular reaction is unlikely to produce balanced flavour. Hence, the need for addition of mixture of enzymes in proper ratio have been advocated. To achieve more intense and balanced flavour in buffalo milk Cheddar cheese within 3-4 months, use of a mixture of 0.001% lipase and 0.01% protease has been suggested (Kanawjia, 1987).

The addition of commercial proteinase preparations led in most cases to a reduction in yield and development of bitter flavour and a cheese with softer body was also reported. Bitter flavour development was considerably reduced after the addition of proteinase/peptidase mixtures.

The major limitations in the use of exogenous enzymes are related to the method of enzyme addition. When enzymes are added to milk, a very small portion is retained in the curd, which increases the cost. Reduction in cheese yield and flavour defects from proteolysis during manufacture and in the early stage of maturation, as well as whey contamination with added enzymes, are major obstacles to the application of this technology. The addition of enzymes to the curd is efficient in case of Cheddar-type cheeses, which enables the addition of enzymes with the salt during curd milling. Hot spots are often noticed due to unequitable distribution of the exogenous enzymes.

**Microencapsulated enzymes:** In order to overcome the problems arising from the addition of free enzymes, two methods for the enzyme entrapment have been evaluated. In the first method, cell free extract from bacteria or whole bacterial cells were encapsulated in milk fat with their appropriate substrates, which led to the release of reaction product in the cheese matrix. The applications described included the generation of diacetyl and acetyl methyl carbinol and of 3-methyl butanal and 3-methyl butanol from leucine. Finally, a system containing Penicillium spores and milk fat leads to the generation of methyl ketones. This approach could be applied to low-fat cheese flavour enhancement and for the production of a flavour enhanced cheese for use in the snack industry.

In the second method, proteinases were entrapped in phospholipids vesicles (liposomes) to protect the milk proteins from the actions of the enzymes during the cheesemaking process, which should limit bitter flavour development and weight
loss. Liposome technology is scientifically attractive method that is widely used in the pharmaceutical industry but may be relatively expensive for the cheese industry. Addition of enzymes to cheese milk leads to a partitioning of the enzyme between curd and whey, representing an economic loss of the enzyme (only 5-10% of enzymes is retained in the curd). Alternative method of direct enzyme addition with the salt at milling is not applicable to brine-salted varieties. Therefore, addition of encapsulated enzymes enclosed in liposomes, such as Flavourage, Naturage and Accelase at the rate of 2.5 g/100 litres of milk was found to accelerate ripening in buffalo milk Cheddar cheese (Rao, 1991).

**Supplementation with goat milk:** It is heartening to note that supplementation of buffalo milk with goat milk (10-25%) improves rheological, textural and sensory properties of buffalo milk cheese and reduces the ripening period. Addition of both goat milk and microencapsulated enzymes exhibited synergistic effect on flavour development.

**References**

**TECHNOLOGY FOR MANUFACTURE OF KRADI**

G.R. Patil

**Introduction**

*Kradi*, also referred as milk bread, is a famous traditional milk product of Jammu and Kashmir (Lawrence, 1886). *Kradi* is the name given by the people of Jammu and Kashmir to a heat and acid coagulated dairy product. It has two more synonymous names viz. *Maush Kraer* (Maush in Kashmiri language means buffalo, therefore, Maush *Kraer* means *Kradi* made of buffalo milk) in Srinagar and *Kalari* in the upper hilly regions of Jammu division of Jammu and Kashmir but the product is identified and familiar with *Kradi* name. It is a type of fresh unripened cheese made by heat coagulation of buffalo milk with some easily accessible coagulating agent like *lassi* (sour buttermilk) and working out the coagulum into a pat. Finally small balls are made out of it, which are later given a circular shape of varying diameters ranging from 5 to 20 cm with a thickness of about 0.2 to 2.5 cm. It is manufactured primarily by the tribal population of Gujjars and Bakarwals living in the hills of Shopian, Kupwara, Pahalgam, Poonch, and Rajouri areas of Jammu and Kashmir, India and Muzzafarabad of Pakistan occupied Kashmir. The Gujjars and Bakarwals are poor and backward sections of the society whose main source of income is from the sale of milk and milk products. *Kradi* making is an art handed down from generation to generation of Gujjar and Bakarwals tribes. The tribal communities in the upper hilly regions and people in some parts of Jammu division of Jammu and Kashmir call this product as Kalari which in urban areas is considered to be a dairy delicacy often being served in feasts and marriage parties to the valued guests. Summer season is considered to be most suitable for its manufacture; however, the product can be prepared throughout the year depending upon the availability of raw material. *Kradi* is well relished by all the sections of the society, particularly by the affluent class. It is a fancy product either eaten raw or used as an ingredient for preparation of several Kashmiri dishes. It is consumed after frying it with some suitable frying medium along with spices and condiments. It is also consumed in the form of a culinary dish in combination with vegetables and gravy. Besides being a salubrious food, *Kradi* is believed to possess antidiarrhoeal, anticold and antitussive properties. This product has a tremendous market potential and is considered a delicacy throughout the state. It is a semisoft, white, unripened cheese. *Kradi* resembles *Ayib*, a soft variety of cheese of Ethiopia made by heat coagulation of buttermilk. The composition of *Ayib* is about 76% water, 14% protein, 7% fat and 2% ash. Although *Kradi* also resembles Ricotta and Mozzarella cheese in certain aspects related to manufacturing procedure, it is altogether a different product having unique characteristics. The uniformity of the product depends on the quality and
composition of milk that is used for its manufacture. It is characterized by high moisture content, however, for preservation it is sometimes sun dried and later consumed in lean season. The *Kradi* available in markets from four diverse regions viz. Shopian, Pahalgam, Rajouri and Poonch of Jammu and Kashmir vary in size and shape, as well as sensory, physico-chemical, functional property, microbiological, instrumental color and textural characteristics. A study revealed that sensorily, the samples from Shopian area were fermented and had glossy top & bottom (Hilal *et al.*, 2007a). The samples from Pahalgam area were curdy, smoky with smooth surface and had a glossy top. The samples from Rajouri area were mealy/ grainy, nutty, bitter, moldy, foreign and unclean, whereas the samples from Poonch area were crumbly, rubbery and pasty. Proximate composition of *Kradi* from different market areas has been given in Table 1.

| Table 1: Proximate composition of *Kradi* from different market areas |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| **Parameter**      | **Market areas**  | **Std Method**    |
|                   | Shopian           | Pahalgam          | Rajouri           | Poonch            |
| Fat (%)            | 28.63             | 22.70             | 6.35              | 7.06              | 5.59              |
| Protein (%)        | 24.08             | 20.96             | 32.37             | 31.27             | 42.91             |
| Moisture (%)       | 44.91             | 54.04             | 58.63             | 59.04             | 49.09             |
| Ash (%)            | 1.64              | 1.66              | 1.26              | 1.34              | 1.09              |
| Lactose (mg %)     | 0.70              | 0.61              | 1.37              | 1.26              | 0.82              |
| pH                 | 5.04              | 5.41              | 5.24              | 5.24              | -                |
| Acidity (%LA)      | 0.51              | 0.57              | 0.56              | 0.55              | 0.53              |
| Fat leakage        | 3.01              | 2.21              | 2.36              | 2.29              | 0.16              |

Wide variation is observed in chemical composition of *Kradi* from markets of different region of Kashmir. The *Kradi* from Shopian and Pahalgam region had high fat and low protein than *Kradi* samples from other two regions. Wide variation was also observed in textural parameters of *Kradi* from different regions of Jammu and Kashmir (Table 2). The Shopian samples were more hard and chewy and Pahalgam samples more adhesive and resilient than other samples.

| Table 2: Texture profile parameters and tensile strength of market samples of *Kradi* |
|-------------------|-------------------|-------------------|-------------------|
| **Parameter**      | **Market Areas**  | **Std Method**    |
|                   | Shopian           | Pahalgam          | Rajouri           | Poonch            |
| Hardness (N)       | 120.26            | 102.67            | 107.74            | 109.46            | 151.54            |
| Adhesiveness (N.s) | -0.47             | -6.23             | -0.04             | -0.33             | -0.004            |
| Springiness (mm)   | 0.92              | 0.78              | 0.74              | 0.98              | 0.75              |
| Cohesiveness       | 0.58              | 0.57              | 0.38              | 0.49              | 0.38              |
| Gumminess (N)      | 71.4              | 56.36             | 48.22             | 57.09             | 49.56             |
| Chewiness (N.mm)   | 66.61             | 53.86             | 42.4              | 50.23             | 52.41             |
| Resilence          | 0.33              | 4.14              | 0.20              | 0.25              | 0.23              |
| Tensile strength (N)| 2.93              | 1.72              | ND                | ND                | 5.12              |
The instrumental colour quality of market samples of *Kradi* revealed that the *Kradi* samples from Pahalgam were darker than other samples (Table 3). The *Kradi* samples from Shopian were found to be best in overall quality followed by *Kradi* samples from Pahalgam, Poonch and Rajouri regions.

**Table 3: Instrumental colour quality of market samples of *Kradi***

<table>
<thead>
<tr>
<th>Parameter (hunter value)</th>
<th>Shopian</th>
<th>Pahalgam</th>
<th>Rajouri</th>
<th>Poonch</th>
<th>Std Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lightness (L*)</td>
<td>77.54</td>
<td>71.64</td>
<td>77.93</td>
<td>76.04</td>
<td>82.93</td>
</tr>
<tr>
<td>Redness (a*)</td>
<td>0.58</td>
<td>-0.77</td>
<td>-0.60</td>
<td>-0.60</td>
<td>-0.23</td>
</tr>
<tr>
<td>Yellowness (b*)</td>
<td>16.73</td>
<td>17.53</td>
<td>15.03</td>
<td>14.8</td>
<td>17.16</td>
</tr>
</tbody>
</table>

The microstructure of the market samples of *Kradi* was also studied using SEM, which revealed that a large mass of fused fat-protein complex with some irregular shaped voids. The fat particles are noticeable in the market samples of *Kradi*. The aggregations and connections between casein and fat globules were well developed. The fat globules were smooth, irregular shaped embedded in the casein matrix.

**Standardization of improved method of manufacture**

There are many limitations of the traditional method of preparation of *Kradi* as the traditional method leads to non-uniform quality. Hence, an attempt was made to standardize a commercially adoptable process for the large scale manufacture of *Kradi* so as to put this very important variety of cheese on the map of traditional Indian milk products. The efforts were directed towards studying the raw material use, processing techniques and end-product characteristics in order to find out the technological lacunae and improve upon them by incorporating modern methodologies of dairy science and technology for the development of product in tune with the national and international standards. The product development work essentially comprised of optimization of levels of fat, bacterial cultures (NCDC-167/NCDC-144), cultured skim milk to milk ratio, pH of coagulation and coagulation temperature using response surface methodology (RSM) designs (Hilal *et al.*, 2007 b,c). The optimized method has been given in Fig. 1. A product having maximum sensory attributes, highest yield, lower acidity, optimum fat leakage, hardness, chewiness and colour parameters could be obtained by using fat level, 1.5-2.5 %; milk to cultured skim milk ratio, 1.36-3.0 ; pH of coagulation, 5.0-5.4; and coagulation temperature, 60-70°C. A patent on the optimized process has been filed. The optimized product developed with culture NCDC-167 was found to be best than the optimized product developed with culture NCDC-144. The compositional parameters, the textural parameters and colour parameters of *Kradi* obtained by the standardized method are given in Table 1, Table 2 and Table 3, respectively.

**Shelf life:** The vacuum packed *Kradi* had shelf life of 20 days at 25°C, 16 weeks at 5°C and more than 6 months at -20°C. The non-vacuum packed *Kradi* had a shelf life of 15 days at 25°C, 14 weeks at 5°C and more than 6 months at -20°C.
Consumer's preference: Consumer study of the optimized product revealed that about 67% of the consumers rated it excellent, 17% of the consumers rated it very good, 9% of the consumers rated it good and 7% of the consumers rated it fair. About suitability of the product for preparation of dishes, 64% of the consumers rated it excellent, 16% of the consumers rated it very good, 12% of the consumers rated it good and 8% of the consumers rated it fair. About suitability of package for the product, 88% of the consumers rated the package good and 12% of the consumers proposed for alternate package.

Cost of production: The net cost per packet of *Kradi* containing 100 gm of product comes to about Rs. 19.53 (Rs. 195.30/kg.). Even if the transport & profit margins are added @ 20 percent of product the cost, total sale price will be Rs. 23.50 per packet.

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![Flow diagram for manufacture of Kradi](image_url)
References


Unprecedented growth in the functional and health foods market all over the world during the last few years has helped Probiotics to re-emerge as a hot commodity due to expressing several health promoting functions in the gut. This rapid growth in demand for probiotic foods is largely attributed to growing awareness among consumers about linkages of diet with general health, discovery of new probiotics with novel health functions and high commercial potential of the market for probiotic foods. It is now recognized that diseases associated with malfunctioning of the human gut and other serious medical conditions can be combated through consumption of probiotic cultures or food formulations. These can be used either prophylactically (preventive) or as biotherapeutics (control) against a particular disease as an alternative to drug treatment. As compared to many pharmaceutical agents, probiotics are well tolerated by human anatomy and are extremely safe. However, probiotics as a concept is still associated with a large body of unsubstantiated claims. There are major concerns regarding quality, efficiency, labeling and so also verification of these parameters. This situation calls for an international consensus on protocols for evaluating the efficacy and safety of these products. There is lack of uniformity in regulatory guidelines for probiotics across nations of the world while many developing countries, including India, are yet to formulate a regulatory framework for probiotics. This makes the task of setting up a globally acceptable evaluation process for monitoring of health claims of probiotic products rather difficult.

**What are Probiotics?**

Food and Agricultural Organisation (FAO)/World Health Organisation (WHO) guidelines define Probiotics as live micro-organisms which, when administered in adequate amounts, confer a beneficial effect on the health of the host. The term 'Probiotic' meaning 'For Life' was first coined in 1965 by Lilly and Stilwell, although the original concept can be traced back to the pioneering work of Metchnikoff in early 1900's.

Two main genera of Gram positive bacteria *Lactobacillus* and *Bifidobacterium* are used extensively as pro-biotics. However, other genera such as *Escherichia*, *Enterococcus* and *Saccharomyces* have also been marketed as probiotics although concerns still remain regarding the safe use of these organisms. Current evidence indicates that probiotic effects are strain specific. Therefore, a beneficial effect
attributed to one strain can not be extrapolated to another strain even when it belongs to the same species.

As per guidelines adopted by the FAO and WHO, probiotic cultures used in human food must be properly identified at genus, species and strain level by well accepted molecular techniques such as PCR, 16s rRNA sequencing and DNA fingerprinting tools viz. Ribotyping, ARDRA, PFGE etc. They must be capable of surviving a passage through the gut, i.e., they must have the ability to resist gastric juices and exposure to bile. Simultaneously, they should be able to proliferate and colonize the digestive tract. Finally, they must be safe and effective and maintain their potency during the entire shelf life of the product. Caco2 cell adherence can be used as an effective in vitro assay system for evaluating the colonization potential of probiotic strains before conducting clinical studies on human subjects.

**Probiotics and Human Health**

Apart from the current application of probiotic bacteria as a general health supplement, an increasing volume of clinical data clearly suggests the effectiveness of probiotics in the treatment of specific diseases. Double blind placebo controlled studies have identified potential benefits of ingesting probiotic organisms in patients suffering from lactose intolerance, rotavirus diarrhea, inflammatory bowel disease (IBD)/irritable bowel syndrome (IBS) and certain types of allergies. Probiotics are also known to exhibit anti-carcinogenic effects by producing antioxidants capable of scavenging free radicals. Probiotics also exert an immunomodulatory role by promoting the endogenous host defense systems, particularly mucosal immunity, by stimulating phagocytosis, macrophages and other immunological markers such as interleukins, TNFα and natural killer cells, etc. They are also known to reduce the risk of vaginitis and other sexually transmitted and urinary tract infections by inhibiting the proliferation of pathogens.

Another potential application of probiotic culture is the production of fermented food products enriched with health promoting substances such as conjugated linoleic acid (CLA). CLA has gained considerable attention in recent years because of its several beneficial effects including anticarcinogenic and antiatherogenic activities, ability to reduce catabolic effects of immune stimulation, ability to enhance growth promotion and the ability to reduce body fat. Of the individual isomers of CLA, cis-9, trans-11-octadecadienoic acid was implicated as the most biologically active and predominant isomer found in the diet. This arises from the microbial biohydrogenation of dietary linoleic acid to CLA in the rumen.

**Probiotics: Importance of dosage**

Adequate number of viable cells, the “therapeutic minimum”, need to be consumed regularly for transferring the “probiotic” effect to consumers. It has been suggested that minimum viable number of $10^6$ CFU ml-1 or gram is required to be ingested in the gut through food or formulation. One needs to consume 100 g of bio-yoghurt
Designing novel functional/probiotic health microbes and foods

As a result of rapid advancements in genetic accessibility and protocols for Lactobacilli, and availability of the complete genome sequences for common probiotic strains, substantial opportunities and possibilities exist for the development of strains with safe and effective health promoting effects. The most notable novel recombinant probiotic at present is a derivative of *Lb. johnsonii* La1. La1 is a well characterized probiotic strain used extensively in commercial preparation of probiotic foods due to its strong health-related attributes and

(yoghurt with probiotics) per day to obtain more than $10^6$ CFU ml-1. According to regulation, bio-yoghurt should contain living bacteria cells $2 \times 10^6$ living bacteria in 1 ml - at the end of the recommended storage period. The daily dose of probiotic microorganisms should reach $1 \times 10^9$ cells. The titer of bacteria in fermented drinks reaches $10^8$ to $10^9$ /ml and decreases with storage. It is also possible to use tablets or capsules that contain lyophilized cultures of bacteria as additives to foodstuffs.

**Mining the probiotic genome**

Recent advances in genomic technologies particularly DNA sequencing have made it possible to accurately decipher the entire complement of probiotic bacteria. The sequencing capabilities have been enhanced through improved computer software that can annotate or identify the majority of genes encoded by the sequence. The genome of a number of probiotic bacteria such as *Lactobacillus gasseri*, *Lb. casei*, *Lb. rhamnosus*, *Bifidobacterium longum* and *B. breve* have already been completed and more are underway. In addition to defining the genetic capability of these organisms, whole genome comparisons of probiotic strains vis-a-vis others will identify unique characteristics of these organisms. Moreover, the DNA sequence data represents only the first critical step in mining the probiotic genome. The availability of genome sequence will be important in defining the capabilities of individual probiotic strains. Since colonization in the hostile environment prevalent in human gut is a very critical step for selection of the probiotic strains in foods or other formulations, it would be interesting to target genes encoding bile salt hydrolase (BSH), surface layer proteins viz. mucin binding protein (MBP), collagen binding protein (CBP) and fibronectin binding protein (FBP) etc. which are associated with adherence on gut epithelial cell lining. Understanding their exact mechanism of action helps in designing strategies to improve the colonization potential of probiotics. These genes can be used as potential probiotic markers for screening of novel probiotics. This data will also form the platform for microarray and proteomics technologies that allow real time analysis of RNA and protein expression in the bacterial cell. Detail investigations of probiotic organisms with these new and potentially powerful tools will facilitate development of bacteria as therapeutic agents and provide the mechanisms to produce novel probiotic strains for application in customized health foods for specific target populations.
positive immuno-modulatory effects on the host. Milk fermented with this culture produce a racemic mixture of D and L-lactate in the ratio 60:40. Presence of D-lactate in milk fermented with La1 and ability of the strain to produce D-lactate after ingestion does not pose any problem to most of the adult population. But it can indeed cause D-acidosis and encephalopathy in patients suffering from bowel syndrome and intestinal failures, and in new born infants with immature liver. However, inactivation of the single copy D-lactate dehydrogenase (LdhD) gene of La1 resulted in rerouting of pyruvate to many L-lactate with no D-lactate production. This novel strain has the same beneficial properties as the parent probiotic while the absence of D-lactate makes it a safer alternative for specific populations.

Amongst several other possibilities is the design of recombinant strains with novel properties that confer competitive advantage to their survival. One way to accomplish this strategy is by expressing and secreting colicin V, a narrow host range antibacterial bacteriocin produced by E. coli in La1 by replacing the original colicin V leader peptide in colicin with a signal peptide from divergicin A of Carnobacterium divergens. This strategy has allowed the expression and secretion of the Gram negative antimicrobial in probiotic organisms to extend their inhibitory spectrum to Gram negative enteropathogens too.

Established probiotic lactobacilli can also serve as attractive candidates for oral vaccination against HIV, tetanus, Rota virus, E. coli, Salomonella and H. pylori etc. in view of their long history of safe use, ease of oral administration, low intrinsic immunogenicity and extensive industrial handling experience. Recently, a series of expression vectors were constructed that allowed secretion of human myelin protein by probiotic Lb. casei into the growth medium. This recombinant strain may be useful in oral tolerance induction for intervention in the auto-immune disease, multiple sclerosis.

**Probiotics: safety concerns**

As probiotic bacteria have to be consumed in large quantities over an extended period of time for ensuring beneficial effects, safety becomes a matter of critical concern. Traditionally used probiotics, particularly lactobacilli in food processing, have long attested to their safety due to absence of any significant adverse effects in the humans. In recent years, there have been reports of isolated cases of opportunistic infections caused by certain probiotics such as Enterococcus and Saccharomyces spp. This is of considerable medical relevance because of their increasing association with nosocomial infection, coupled with evolving vancomycin resistance. However, the potential for genetic transfer of virulence factors from medical strains to culture starter strains via a natural conjugation process has now been demonstrated. Current FAO/WHO guidelines recommend that probiotic strains should be evaluated for a number of parameters, including antibiotic
susceptibility patterns, toxin production, metabolic and haemolytic activities, ineffectivity in immuno-compromised animal models, side-effects and adverse incidents in humans.

**Application in functional foods**

*Functional foods* as a marketing term was initiated in Japan in the late 1980s and is used to describe foods fortified with ingredients capable of producing health benefits. This concept is becoming increasingly popular with consumers because of a heightened awareness of the link between health, nutrition, and diet. Food manufacturers are enthusiastic about developing such products because the added ingredients give increased value to food. The global market for functional foods in the coming years is predicted to grow rapidly. Although Japan currently accounts for about one-half of this market, the fastest rate of growth is expected to be in the United States. In Japan functional foods are considered a major product opportunity and > 80 recognized functional foods are available. In 1991 functional foods were given legal status in Japan, where they are described as FOSHU, indicating foods for specific health use. A FOSHU is defined as a food expected to have certain health benefits and that has been licensed to bear a label to that effect. Proven scientific evidence of the health effect is a prerequisite to obtaining FOSHU status. It is worth emphasizing, however, that the health claims are limited to health maintenance and not the curing of disease. Functional foods as a category have enjoyed considerable growth and are now firmly established, with >300 firms operating in Japan.

In Europe, interest in functional foods has increased over the past several years as the market outside of Japan has developed. In particular, probiotic foods are now relatively well established in Europe and product activity during 1997 consisted of a more concentrated launch of prebiotic foods, particularly in the dairy sector. Although probiotic foods seem to be reasonably well established in Europe and Japan, they are typically considered niche products in the United States. It is expected that product development in functional foods could outstrip development in low-energy and "light" foods, which was a key area of growth in the early 1990s.

**Probiotics in India: Current status**

A radical change is now evident in the country in terms of introducing probiotics for value addition in our ethnic foods. Dairy products, including yoghurt, cheese and ice-cream and traditional foods like Dahi, Srikhand and Kulfi remain at the forefront of probiotic food development in India. Yoghurt with added live probiotic strains is now available commercially. India is fast emerging as a potential probiotic market of branded yoghurt/curd and cultured milk/lassi with probiotics, both growing at a rate of 14 percent and 33 percent respectively.

Even though branded probiotic product manufacturing is at a nascent stage in India, a survey conducted by M/s Euromonitor International concludes that considerable amount of work has been done in the country on probiotic cultures from different
perspectives. Yet, hardly any attempt has been made to tap the enormous biodiversity and uniqueness of indigenous microflora of probiotic lactic acid bacteria available in the country for commercial exploitation. In this context, a DBT sponsored Network project on Probiotics was taken up at National Dairy Research Institute (NDRI), Karnal in collaboration with All India Institute of Medical Sciences (AIIMS), New Delhi; and National Centre for Cell Sciences (NCCS), Pune, with the objective of establishing a national repository of indigenous probiotic cultures/germ plasm as well as validation of health claims by clinical trials in human subjects. NDRI has already taken a lead in establishing a repository of well characterized and catalogued indigenous probiotic lactobacilli with strong colonization potentials and novel physiological functions.

The availability of genome sequences of several popular probiotic strains has led to a renewed interest in these probiotic cultures. Indigenous strains of probiotic cultures can play a vital role in human health care and functional health foods market. They can provide an effective alternative to pharmaceutical drugs for prevention and treatment of serious medical conditions such as gastrointestinal infections and diarrhoea, genitourinary infections, allergies, certain bowel disorders and lactose intolerance, etc. which afflict a considerable proportion of the global population. However, considerable work is required to ascertain specific health benefits adequately supported with well designed clinical studies to draw clear cut conclusions regarding the efficacy of selected probiotic strains/foods. In this presentation, the major R&D leads on different aspects of probiotics particularly indigenous lactobacilli of human gut origin and their prospects as dietary ingredients and biotherapeutics for promoting health and gut immunity along with management of specific inflammatory metabolic disorders will be the main focus and discussed at length.
Feta cheese is a semi-soft, white-brined cheese traditionally made from Sheep's milk or from mixture of Sheep's and Goat's milk. Its typical flavour is mildly rancid, slightly acid and salty. It has a rather firm and smooth texture, which makes the cheese sliceable. No gas holes should be present, but irregular small mechanical openings are desirable (Abd El-Salam, et al., 1993). Feta cheese was made from thermized milk with no starter at small scale production. Pasteurized milk and a yoghurt culture, or a combination of mesophilic and thermophilic lactic acid bacteria, are used for larger-scale production (Tammie and Kirkegaard, 1991).

The Feta cheese is one of the most popular white-brined cheese, which is nowadays globally manufactured on an industrial scale. It is rindless, white, soft and salty, originally made from sheep milk, goat milk or mixture of two. It is very popular in Greek, Yugoslavia, Bulgaria and middle-east countries. There is a growing demand for this cheese in middle-east countries. In European countries, techniques have been developed to manufacture Feta Cheese from cow milk. Since the typical character of cheese is white colour, cow milk has to be bleached to result in desired colour. Blending destroys valuable β-carotene. As such buffalo milk can be an ideal substitute to sheep milk which is dwindling in the world. Technology for the manufacture of feta cheese from buffalo milk has been developed at Cheese and Fermented food lab of National Dairy Research Institute, Karnal.

Technology

Milk is standardized according to type of milk. Then the standardized milk is pasteurized and cooled in a vat according to type of culture to be used. Generally mesophilic cheese culture and yoghurt culture @1% to 2% are used according to type of milk to be used and cool to 30-35°C and 42-47°C respectively. Subsequently, milk is renneted at 32°C and coagulation takes place in 30-50 min depending upon type of milk. The curd is cut into approximately 1-2 cm³ cubes with vertical and horizontal knives and left for 10-20 min for removal of whey and then curd is transfer into moulds lined with muslin cloth, for further removal of whey and texturization of cheese curd without pressure. Whey is collected and stored at 5°C for making brine. Moulds were turned every 2-4 hour to form a firm curd. After four-five turns, the moulds are left undisturbed for about overnight. Subsequently, cheese blocks are cut into uniform size and transfer to the whey brine solution for about 22-24 hours. Then, cheese cubes are transfer into plastic packages/containers/barrels or tins with or without whey brine for maturation at 10°C for at least 1 month.
Characteristics of starter cultures required in Feta cheese production

Starter to be used

- should have high acid production ability,
- should produce good taste and smell in desired dose and combination,
- should not have high proteolytic activity in order to avoid fast ripening and bitterness,
- should have high antagonistic activity to inhibit pathogens,
- should be resistant to phages,
- should have resistance against antibiotics,
- should grow at cheese production temperature,
- should be resistant to certain salt concentration

General composition

<table>
<thead>
<tr>
<th>Moisture %</th>
<th>Fat-in-DM %</th>
<th>Total protein %</th>
<th>Lactose %</th>
<th>Ash %</th>
<th>Salt-in moisture %</th>
<th>Acidity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>54.34</td>
<td>49.76</td>
<td>17.59</td>
<td>0.53</td>
<td>4.12</td>
<td>4.39</td>
<td>1.86</td>
</tr>
</tbody>
</table>

(Source: Market Survey of Greek Ministry of Commerce)

Process of manufacture

Various methods have been used for manufacture of feta and feta type cheese from different types of milk. Nowadays the demand is increasing day by day for this cheese. Therefore, Feta and feta types cheese are being manufacture by employing ultrafiltered (UF) milk on industrial scale. Few methods are being mentioned here.
Heat treatment 65°C/30min
Cooling (32-35°C)
Addition of Starter culture @ 1-3%
Addition of CaCl₂ @ 0.02%
Renneting 20-30 ml/100L or 2g/100L, 50-150 min
Cutting of Curd (2-3 cm cubes)

Flow diagram of manufacture of Feta cheese from buffalo milk

Buffalo milk
Standardization
Heat treatment
Cooling
Addition of starter Culture

Brining
Vacuum packaging
Ripening & Storage

(Source: Sanjeev and Kanawjia, 2009)
Defects in Feta & Feta type white brine cheeses

Although the secondary microflora might make a beneficial contribution to the development of cheese flavour, components of the same microflora can, on occasions, cause defects.

The most common defect of feta and similar cheeses is 'early blowing', a defect that is characterized by the presence of large gas holes in the cheese, which, in addition, has a spongy texture; this defect is due to coliforms and/or yeasts growing in excessive numbers. However, the problem is rare in modern dairies, provided that efficient pasteurization and good manufacturing practices are applied. Furthermore, the activity of the starter is crucial in the control of coliforms by decreasing the pH and the amount of lactose in the curd.

The presence of coliforms in cheese, particularly *Aerobacter aerogenes*, has been reported to be responsible for blown tins of domiati cheese. A salt content in the milk of 90 g L\(^{-1}\) can prevent this defect. *Klebsiella aerogenes* was found to be responsible for early blowing and poor cheese quality in other white-brined cheeses.

Excessive yeast growth will cause softening of cheese, a condition that is usually associated with an unpleasant yeasty or ester-like odour or gas formation; in the case of white-brined cheeses, swelling of the cans can be caused by yeasts that ferment lactose, e.g. *Kluyveromyces* spp. Discoloration of the surface of a Portuguese ewes' milk cheese has been attributed to pigment-producing yeasts. In addition, yeasts can increase the pH of the cheese surface, thus spurring the growth of *Staphylococcus aureus* and possibly other pathogenic and/or spoilage bacteria. For feta stored over a year, a definite deterioration of quality was noticed when the pH of the cheese increased to more than 5.0.

'Late blowing' is another defect in cheeses and this problem is usually attributed to hetero-fermentative LAB or species of clostridia (e.g. *Clostridium butyricum* and *Clostridium tyrobutyricum*). Although the latter group are sensitive to acid and salt and are more usually associated with problems in Swiss-type and Dutch-type cheeses, some strains of *Cl. tyrobutyricum* are acid-resistant (growing well in a 4.5-7.5 pH zone) and relatively salt-tolerant (tolerating as much as 55-60 g L\(^{-1}\) NaCl at their optimal pH). *Cl. tyrobutyricum* can ferment lactate with the production of butyric acid, which can give an unpleasant aroma at high concentrations, together with carbon dioxide, and it can also generate hydrogen. A similar mixture of gases, released by *Bacillus subtilis*, *Bacillus fastidiosus*, *Bacillus pumilus*, *Bacillus firmus*, *Clostridium paratrificum* and *Clostridium tertium*, was responsible for the swelling of cans of feta cheese.

The development of moulds causing visible defects has been reported for various cheeses, but because white-brined cheeses are stored in tins filled with brine, the development of moulds is rare, provided that the cheese blocks are completely immersed in the brine.
The growth of psychrotrophs might cause certain defects. More specifically, lipolysis might lead to the excessive formation of free fatty acids (FFAs) and rancid flavours in Feta and telme cheese; this is usually the result of contamination of milk with psychrotrophic bacteria, which produce heat-resistant lipolytic enzymes. *Pseudomonas* spp are the most important group of psychrotrophs associated with cheeses; these produce heat-resistant extracellular proteolytic and lipolytic enzymes, which can cause off-flavours and texture defects.

The presence of slime in the brine is a common defect caused by strains of *Lb. plantarum* and/or *Lb. casei* ssp. but it can be prevented by ensuring that the pH of the brine is ~4.0 and the salt content higher than 80 g L⁻¹. Chomakov reported that strains of *Lactobacillus plantarum var. viscosum* were responsible for the formation of ropy substances in the brine of white-brined cheeses. These observations highlight the point that the selection of lactobacilli for use as 'adjunct cultures' in the manufacture of Feta and similar white-brined cheese must be based on an extensive study of their biochemical activities, as the development of desirable flavours or defective products seems to be strain rather than species dependent.

Although most defects are caused by the development of undesirable microorganisms, some chemically driven defects have been reported. For example, the addition of sorbic acid in feta cheese has been reported to produce 1,3-pentadiene, which gives an unclean odour to the cheese.

**Conclusion**

India is largest milk producer in the world producing 109 million tones, out of which more than half (57 percent) of the total milk production are from buffalo milk. There is a great scope for export of feta cheese to middle-east country as European countries has captured the market of middle by developing the technique to manufacture feta cheese from cow milk. Most of the Indian prefer its mild acidic flavour which makes this cheese more popular even in India. More works are to be done for further improvement in the quality of feta cheese.

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• Milchwissenschaft Milk Science International Accepted


Mozzarella cheese was originally manufactured from high fat buffalo milk in the Battipaglia region of Italy, but it is now made all over Italy, in other European countries and USA from cow milk. It belongs to the cheese classified as "pasta filata" which involves the principle of skillfully stretching the curd in hot water to get a smooth texture and grain in cheese. It is a soft, white un-ripened cheese which may be consumed shortly after manufacture. Its melting and stretching characteristics are highly appreciated in the manufacture of Pizza where it is a key ingredient.

The method of manufacture of Mozzarella cheese, irrespective of the milk system from which it is made involves (1) optimum addition of starter culture or proper acidification of milk, (2) renneting of milk, (3) cutting the curd at the right firmness, (4) stirring and cooking the curd particles to the correct consistency and (5) proper cheddaring, stretching and salting of curd for optimum plasticity and elasticity.

Chemistry of "stretch" of Mozzarella cheese

In the calcium rich environment of milk, the casein precipitates out of milk as dicalcium paracaseinate, entrapping fat, insoluble minerals and some sugar. At a pH between 5.2-5.4, resulting from the development (or direct introduction) of acid, some of the calcium of the dicalcium paracaseinate gets dissolved, leading to the formation of monocalcium paracaseinate. This when heated to 54°C or higher becomes smooth, pliable and stringy and retains fat. If acidification is excessive, generally below pH 5.2, monocalcium paracaseinate will continue to lose calcium and from paracasein, which may stretch, but has difficulty in retaining fat. The curd generally does not stretch above pH 5.6.
Manufacturing steps (Traditional method)

Milk

Filtration / Clarification

Standardization (3-4% fat)

Pasteurization (63°C/30 min)

Cooling (31°C)

Starter addition

Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (1:1) @ 1-2%)

Rennet addition (1.0 - 1.5 g/100 l.milk)

Cutting

Cooking (42-44°C)

Pitching

Draining

Cheddaring (0.70% acidity)

Milling

Plasticizing / stretching under hot water (80-85°C)

Molding

Brining (20-22% chilled brine)

Packaging

Storage
Manufacturing Steps (Direct Acid Method)

Milk

Filtration/Clarification

Standardisation (3-4% fat)

Pasteurization (63°C/30min.)

Chilling (4-8°C)

Acidifying
(to pH 5.4-5.6 with 25-50% HCl @ 2.0-3.5 ml conc. acid/l.milk)

Heating (28-30°C)

Rennet addition (0.5-0.75 g/100 l.milk)

Cutting

Cooking (37-39°C)

Draining

Plasticizing/stretching under hot water (80-85°C)

Molding

Brining (20-22% chilled brine)

Packaging

Storage

Advantages of the Direct Acidification Technique

• Curtailed manufacturing time and expenses
• Simplified technology due to elimination of propagation and maintenance of starter cultures
• Starter failures due to bacteriophages and antimicrobial agents avoided
• Less rennet required
• Amenable to mechanization

Disadvantages

• Slight reduction in yield of cheese
• Bland flavor
An ideal Mozzarella cheese has a smooth surface with a perfect sheen, elastic, stringy body free from mechanical openings.

**Chemical Composition**

<table>
<thead>
<tr>
<th>(%)</th>
<th>Mozzarella</th>
<th>Low moisture Mozzarella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>18.0</td>
<td>23.7</td>
</tr>
<tr>
<td>Moisture</td>
<td>54.0</td>
<td>47.0</td>
</tr>
<tr>
<td>Total solids</td>
<td>46.0</td>
<td>53.0</td>
</tr>
<tr>
<td>Protein</td>
<td>22.1</td>
<td>21.0</td>
</tr>
<tr>
<td>Salt</td>
<td>0.70</td>
<td>1.00</td>
</tr>
<tr>
<td>Ash</td>
<td>2.30</td>
<td>3.00</td>
</tr>
<tr>
<td>pH</td>
<td>5.20</td>
<td>5.30</td>
</tr>
</tbody>
</table>

**Defects**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Defect</th>
<th>Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Marbling</td>
<td>Incomplete stretching or mixing, too low water temp., low acidity of curd or a combination of these</td>
</tr>
<tr>
<td>2</td>
<td>Poor melt ability</td>
<td>High salt content</td>
</tr>
<tr>
<td>3</td>
<td>Discoloration</td>
<td>High salt content</td>
</tr>
<tr>
<td>4</td>
<td>Yoghurt flavour</td>
<td>High acetaldehyde production</td>
</tr>
<tr>
<td>5</td>
<td>Browning defect on Pizza top</td>
<td>Use of Streptococcus salivarius subsp. Thermophilus that do not ferment gelatos</td>
</tr>
<tr>
<td>6</td>
<td>Abnormal gas prod., changes in texture and aroma</td>
<td>Propionibacterium freudenreichii</td>
</tr>
<tr>
<td>7</td>
<td>Pigmentation, hole formation, texture changes, off-flavour and aroma in direct acid cheese</td>
<td>Pseudomonas, Achromobacter, Acinetobacter, Citrobacter, Enterobacter, Escherichia, Serratia</td>
</tr>
<tr>
<td>8</td>
<td>Superficial red brown marks, putrid smell, distinct bitter flavour</td>
<td>Pseudomonas putida, P. flourescens, P. palloroni</td>
</tr>
<tr>
<td>9</td>
<td>Soft body → poor slicing, melting</td>
<td>Lactobacillus casei</td>
</tr>
</tbody>
</table>

**Mozzarella Cheese**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Type of Mozzarella</th>
<th>Moisture %</th>
<th>FDM %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mozzarella</td>
<td>52-60</td>
<td>445</td>
</tr>
<tr>
<td>2</td>
<td>Low moisture</td>
<td>45-52</td>
<td>445</td>
</tr>
<tr>
<td>3</td>
<td>Part Skim</td>
<td>52-60</td>
<td>&gt;30 &lt;45</td>
</tr>
<tr>
<td>4</td>
<td>Low moisture part skim</td>
<td>45-52</td>
<td>&gt;30 &lt;45</td>
</tr>
</tbody>
</table>
**Process development for Mozzarella type cheese using skim milk and vegetable oils/fat replacers**

At NDRI studies were carried out to develop good quality Mozzarella type cheese with good stretch, melt, sensory and textural attributes, using cow and buffalo skim milk and blending with vegetable Oils (Soybean oil, Groundnut oil and sunflower) and/or vanaspati. The vegetable oils were mixed with skim milk to give the desired level of fat per cent and this filled milk was converted into Mozzarella type cheese by employing traditional starter culture method. Further, technology developed for Processed Mozzarella enriched with fibres.

- Low Cholesterol Mozzarella cheese variants developed for health and wellness
- Excellent Stretch & Melt properties, highly suitable for Pizza
- Versatile uses
- Nutritionally sound for people of different groups
- Low cost of cheese
- Excellent outlet for use of valuable skim milk solids
- Alleviation of the butter fat scarcity
- Use of fat replacers
  - Starch based: Novagel
  - Protein based: Simplesse
Quarg – the proper German name is Speisequark – is a natural, unripened, fresh cheese produced on a large scale in Germany and is very popular there. It is essentially a milk protein paste, manufactured by acid coagulation of milk by proper bacterial cultures (e.g. *Streptococcus cremoris* and *Leuconostoc citrovorum*) with a small rennet addition for better separation of the protein coagulum from the whey and thus better yields. It is produced in a variety of fat contents, ranging from an essentially fat-free type to a variant with as much as 40 per cent fat in the dry matter. This cheese is popular in central Europe (e.g. Germany, Poland and Austria). Other names for this type of product in different countries include kvarg, tvarog, tworog, twarog, Sauermilchquark and Speisequark. Chakka and Shrikhand are the products related to quarg popular in India.

**Characteristics of quarg**

Quarg is milky white in color, may be even faintly yellowish. Body and texture are homogeneously soft, smooth and mildly supple or elastic. Spreadability must be good. Due to high moisture content (~ 82%, w/w), the shelf-life is limited to 2-4 weeks at <8°C. There should be no appearance of water or whey, dryness or graininess, bacteriological deterioration, over-acidification or bitter flavour during storage (Kroger, 1980; Siggelkow, 1984; Guinee *et al.*, 1993). Odor and taste, i.e. the flavour, must be clean and may be mildly acidic. Quarg is essentially coagulated, flocculated casein with high water content. It is manufactured from milk by acid coagulation and/or rennet coagulation and subsequent separation of whey. The composition is variable and depends largely on the composition of vat milk.

In comparison to most ripened cheeses, Quarg is low in dry matter (DM) and, hence, low in fat and protein and high in lactose/lactate. As most of the calcium is solubilised during the acid coagulation and removed with the whey, it is much lower in calcium than rennet-curd cheeses (Schulz, D. *et al.* 1999).

**Legal requirements**

In the “cheese ordinance” of the German food law, quarg is included under an “umbrella term”- fresh cheese. The product denoted as Quarg must contain at least 18%, w/w, DM, at least 12%, w/w, protein and a maximum 18.5%, w/w, whey protein in the total nitrogen content; products with a DM < 18%, w/w, are to be labelled as Frischkase (Fresh Cheese; Anon, 1986). In other countries, definitions are less stringent or nonexistent. Often, only total moisture and protein contents are
specified, as for, e.g., Kwark or Verse kass (Quarg or Fresh cheese) in The Netherlands.

**Method of manufacture**

High quality quarg can only be made from high quality milk. Therefore, when strong tasting cattle feed has been used, additional laboratory tests are necessary to check the taste, and both acidulating and fermenting qualities of the skim (Siggelkow M.A., 1984).

The manufacture of quarg typically involves pasteurization (72°C for 40 sec) of cheese milk, followed by cooling to 28-30°C. The milk is then coagulated with a mesophilic culture and a small amount of rennet within ~ 16 h. Rennet (~2-20ml standard strength rennet/1000 ltr. of milk) is usually added approximately 90 min after culture addition at a pH around 6.3. The coagulated skim milk is then stirred for ~10-15 min and passed through a tubular strainer to remove curd particles. After separation (34-40°C), the Quarg is cooled, optimally blended with cream or other condiments and packed. The whey discharged from the separator still contains nearly all, i.e., ~0.65%, w/w, whey proteins and 0.2%, w/w, NPN (Siggelkow, 1984; Ramet, 1990; Dolle, 1977; Lehmann et al., 1991; Senge, 2002a).

Whey proteins in the native, undenatured state do not gel under the heating and acidification conditions used in standard separator Quarg production. Various methods have been developed to increase the whey protein content of quarg and reduce losses in the whey.

(i) **Westfalia Thermoprocess**, where, (a) the milk is pasteurized at 95-98°C for 2-3 min to denature and co-precipitate the whey proteins onto the caseins, (b) the resulting finer milk coagulum after fermentation requires a further heat treatment at ~60°C for 3 min (so-called thermization) in order to enhance aggregation and improve sedimentation characteristics. The stirred curd is then cooled (25°C) to a separation temperature. (Dolle, 1977; Kroger, 1980; Siggelkow, 1984; Jelen and Renz-Schauen, 1989; Ramet, 1990; Lehmann et al., 1991). This process gives recovery of 50-60% of the whey proteins in the cheese;

(ii) **Centriwhey Process**, where Quarg whey is heated to 95°C to precipitate the whey proteins. The denatured whey proteins are recovered by centrifugation as a concentrate (12-14% solids) which is added to the milk for the next batch of Quarg (Dolle, 1977, Kroger, 1980; Jelen and Renz-Schauen, 1989);

(iii) **Westfalia Lactal Process**, where the whey is heated (95°C) to precipitate the whey proteins which are allowed to settle; by partial decantation of the serum, a concentrated whey of ~7-8% solids is obtained; this is further concentrated, using nozzle centrifuge (Quarg separator), into a whey Quarg.
(17-18% solids) which is blended at a rate of ~10% with regular Quarg (Dolle, 1977; Kroger, 1980; Jelen and Renz-Schauen, 1989);

(iv) Ultrafiltration (of the gelled milk) is now being used on a large scale for the commercial production of Quarg and other fresh cheese varieties (Patel, Reuter, et al, 1986; Herbertz, 1982; Knupfer, 1982; Kreuder and Libermann, 1983). To produce UF Quarg, acidified skim milk (pH 4.6) is heated to around 40°C and ultra- or micro-filtered to the desired DM content, cooled, optionally homogenised and packed (e.g., Baurle et al., 1984; Siggelkow, 1984; Dieu et al., 1990; Korolczuk and Mahaut, 1991a,b; Rogenhofer et al., 1994; Ottosen, 1996). This method gives complete recovery of whey proteins in the cheese; however, the non-protein nitrogen, which amounts to 0.2-0.3% (w/w) of the milk, is lost in permeate. As native whey proteins are not retained during microfiltration, the curd is usually heat-treated (thermised) before separating the curd from the whey (Dieu et al., 1990). Thermisation of the curd (60°C for 5 min) before ultrafiltration also considerably reduces the development of stale, bitter and metallic flavours (Sachdeva et al., 1993; Rogenhofer et al., 1994). Ultrafiltration is carried out around 40-45°C in order to maintain good calcium solubility so as to remove calcium in the permeate (Ottosen, 1996). Quarg, ultrafiltered at higher temperatures, are described as gritty, granular and coarse (Baurle et al., 1984; Tamime et al., 1991a,b; Sachdeva et al., 1993). Ultrafiltration using mineral membranes was found to be best for making Quarg by UF from fully acidified skim milk (Sharma et al., 1992a; Sharma and Reuter, 1993).

Pfalzer and Jelen (1994) enriched cheese milk with 25% sweet whey UF retentate containing 12%, w/w, DM and 4%, w/w. Protein for an experimental Thermoquark-type fresh cheese produced using cheesecloth bags without significantly affecting the quality of the final product.

In Germany, UF Quarg is used only for Speisequarkzubereitungen (Quarg preparations), as the possible slightly bitter flavour at the end of the shelf-life in plain Speisequark is not satisfactory.

(v) In the FML process (Forschungsinstitute fur Milch und Lebensmittel, Weihenstephan), skim milk is nanofiltered 2 fold to 7%, w/w, protein and then fermented. The coagulum is stirred and concentrated by either ultrafiltration or separation. A separator needs to be adapted to the higher viscosity of the retentate coagulum in comparison to unconcentrated fermented skim milk. The texture of the final product is between that of conventional UF fresh cheese and of Thermoquark (Schkoda and Kessler, 1996, 1997a, b). Muchetti et al., (2000) confirmed the findings of Schkoda and Kessler by nanofiltering milk 2.1-fold.

(vi) In Aubios process, Hannover, the skim milk is pre-concentrated 1.7-fold to 5.4%, w/w, protein (or upto 2.2-fold without causing bitterness) using microfiltration, producing a product which is similar to Thermoquark
(Hulsen, 2002). A special combination of starter cultures is needed for the fermentation of retentates as more lactic acid must be formed than in unconcentrated milk.

**Quarg filtration technology**

Filtration technology can be used at different stages during the manufacture of Quarg-type products, e.g., filtration of the acid whey, (partial) filtration of the sweet milk or filtration of (partially) acidified milk. The yield is higher than for Thermoquark as all whey proteins are incorporated. However, the structure is different from conventional Quarg as UF Quarg is generally softer, smoother and creamier. This can be an advantage if consumed as such; however, for cheese-cakes or desserts, the higher firmness of conventional Quarg is more desirable.

When full filtration to final cheese solids was carried out before acidification, the sensory attributes of the resulting products were described as impaired due to bitterness contributed by the slower rate of acidification, failure to reach the desired pH and the high calcium content (Dolle, 1977; Kroger, 1980; Kreuder and Liebermann, 1983; Baurle et al., 1984; Mann, 1984; Patel et al., 1986). This problem has been overcome by UF of partially (pH 5.7-5.95) or fully (pH 4.8-4.6) acidified milk. Table 2.1 summarises the yield and whey protein recovery for the various methods.

**Table 2.1: Yield of quarg and whey protein recovery using various production methods for quarg cheese**

<table>
<thead>
<tr>
<th>Method</th>
<th>Principle</th>
<th>Typical Yield (kg skim milk/kg Quarg)</th>
<th>% Whey protein recovery in Quarg</th>
<th>Flavour and Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Westfalia Standard Separator Process</td>
<td>Separation of acidified milk</td>
<td>4.50-4.70</td>
<td>~15</td>
<td>Firm and sour</td>
</tr>
<tr>
<td>Westfalia Thermo-compressor</td>
<td>Separation of acidified milk</td>
<td>4.08-4.30</td>
<td>50-70</td>
<td>Firm, smooth and mild</td>
</tr>
<tr>
<td>Centrywhey/Westfalia-Lactal/Meggle-Alcor</td>
<td>Separation/Decanting/Ultrafiltration of whey</td>
<td>3.98</td>
<td>50-100</td>
<td>Whey taste possible</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>Full UF of milk</td>
<td>~3.8</td>
<td>~100</td>
<td>Bitter taste</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>Full UF of acidified milk</td>
<td>3.60-3.98</td>
<td>~100</td>
<td>Smooth</td>
</tr>
</tbody>
</table>
Weihenstephan (FMLa) process
Nanofiltration of milk to a volume concentration factor (VCR) of 2. UF or separation of acidified retentate ~ 3.4 < 100 Smooth, sweet mild flavour

Hannover (aubios) process
Microfiltration of milk (VCR = 1.7-2.2). Separation of acidified retentate 4.00 < 60 As Thermoquarg

(Research institute for dairy and food), Weihenstephan, Germany

Data from Anon, 1984; Baurle et al., 1984; Lehmann et al., Schkoda and Kessler, 1997; Hinrichs, 2001; Hulsen, 2002)

**Equipments involved in modern manufacture of quarg cheese**

Concentration to the desired dry matter content for quarg (17-20%) and a protein content of 12.5-13.5% (for low-fat quarg) or separation of gel into whey and quarg can be done by the following processes:

- Batch separation by pressing or drainage by using filter cloth or filter bags
- Continuous centrifugation using quarg separators
- Continuous concentration or separation by ultrafiltration

**Technology developed at NDRI for manufacture of quarg cheese**

Quarg cheese was manufactured by using method described by Spasenija D. Milanovic et al. (2004) with some modifications. Good quality standardized buffalo milk (Fat- 3.0±0.30%, SNF- 9.50±0.25%) was taken in a vat and pasteurized at 85°C for 15 minutes. The cheese milk is then cooled and inoculated with NCDC culture @ 1.0 percent and incubated at temperature 37°C. Two and half hrs (2.5 hrs) after the addition of starter culture (when the pH reaches up to 6.3), Meito rennet @ 200-300mg/100kg milk was added and mixed thoroughly. Thereafter the vat content was left undisturbed for curd setting, which took around 14-16 hrs starting from culturing. The coagulum was then cut using 1/3 inch cheese knives and it was again left undisturbed for about 10-15 minutes. The temperature of the contents was then slowly and gradually increased to 55-60°C @ 1°C per minute and the curd kept hold for 10 minutes at 60°C as per the requirement for thermoquarg manufacture. Cooked curd was then cooled to room temperature and filled in muslin cloth for 3.5 to 4 hrs (TS around 28-30%) for dewheying. Thereafter fibers, plant sterol esters and salt were added in curd and homogenization of total mass was carried out in Hobart mixer. The quarg is then filled in PS cups and stored at 6±1°C.
Enhancement of functional attributes in Quarg Cheese

The current trend is functional foods development is to enhance the health attributes of widely consumed foods by fortifying with functional ingredient. It has been established that plant sterols and stanols lower blood cholesterol levels by partly blocking absorption of cholesterol in the gut. Now they are widely available in a range of food products for those who want to lower their cholesterol level. Dietary fiber is another food ingredient gaining lot of attention from the health point of view.

Enrichment with dietary fibers

Dietary fiber, especially soluble fibers are associated with carbohydrate and lipid metabolism has shown to have hypercholesterolemic properties. Keeping in view the reported beneficial effect of dietary fiber on cardiac disease, inulin (Raftiline), oat (Vitacel) fiber and soy fiber were assessed for their suitability. Inulin was used @ 8-12%, w/w, of curd and oat fiber and soy fiber @1-3%, w/w, of the curd in order to provide sufficient concentration of dietary fiber in the product. The study revealed addition of oat fiber at the level 1.0 % resulted in an increase in all the sensory scores of all attributes studied and also found very close to control. Further increasing the level of oat fiber from 2.0 to 3.0 percent, there was substantial decrease in the sensory scores. Oat fiber @ 1.0 percent showed the highest overall acceptability may be because of reduced free whey, whitish to creamy colour, good body and textural attributes. The lowering of scores for body and texture at higher levels of oat fiber could have been because of harder body, creamy colour and poor spreadability of the product. Sensory responses of quarg cheese with different levels of soy fiber inferred that the quarg cheese containing 1.0 percent soy fiber received highest flavour, body and texture, colour and appearance as well as overall acceptability score. Further increasing the level to 3.0 percent resulted in significant (p<0.05) lowering of sensory scores. Inulin is recognized to act as fat mimetic and to improve textural characteristics of the product. Addition of inulin can effectively bind water in high moisture content system and prevent phase separation. The evaluation and selection of level of inulin was based on sensory parameters. These results reveal that the quarg having 10.0 percent inulin was liked very much by judges. Further increasing the level to 12.0 percent resulted in significant (p<0.05) lowering of sensory scores for all attributes. Also, the product hardness was increased too much during storage, along with reduced spreadability. In addition, inulin made the product slightly sweetish.

Optimization of level of incorporation of plant sterol esters

In the present study, effect of different levels of plant sterol esters i.e. 2, 3, 5 and 7 per cent, on the quality of fiber enriched quarg cheese was studied with the aspiration to explore the enrichment of quarg cheese with plant sterol esters. The
study demonstrated that adding plant sterol ester had no significant change in sensory quality of fiber enriched quarg cheese.

**Enrichment of Quarg cheese with prebiotic and probiotic attributes**

The technology was designed to develop the probiotic Quarg cheese with enhanced therapeutic dose for claimed health benefits up to the shelf life of product. Two selected probiotics added in Quarg cheese in three different forms viz. propagated probiotic culture, concentrated cell biomass and encapsulated probiotic at two different stages viz. with traditional starter culture (TSC) and at mixing stage, and results of various parameters compared with control sample. In each process prebiotic inulin was also added for stimulation of growth of probiotic. Probiotic Quarg cheese manufactured by each process was evaluated for the sensory, textural, physico-chemical and survivability of probiotic in fresh cheese sample as well as during storage also. Based on these results method of manufacturing and probiotic culture were selected for further storage study. The results obtained revealed that probiotic Quarg cheese with desired quality attributes and therapeutic dose can be made employing M3 and M4 methods. Further, it is inferred that M2 method has adverse effect on quality attributes of Quarg cheese. It was also observed that Quarg manufactured using probiotic *L. casei* (NCDC 298) possessed good overall acceptability and survivability during storage of 30 days.

**Extension of shelf life**

Quarg cheese has a shelf life of about 2 week under 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 the trials were undertaken to extend the shelf life of quarg cheese using MicroGARD™ 100 (0.15, 0.30 and 0.50 per cent) and Nisin (200 IU and 250 IU). The product was packaged in polysterine cups and stored at refrigeration temperature (6±1°C) and evaluated for sensory, physico-chemical and microbiological attributes at predetermined intervals. The study revealed that use of MicroGARD™ 100 or Nisin could be successfully practiced to extend the shelf life of the Quarg cheese without adversely affecting the quality of Quarg cheese.

**Future outlook for quarg cheese**

In northern Europe, the market is expected to expand. Economists, nutritionists, dieticians and many medical practitioners have recommended quarg consumption actively and will undoubtedly continue to do so. Even hotel have now begun to give it in breakfast. As for the American market, it’s anybody’s guess. Quarg is virtually known now in the USA. European immigrants ask for it but don’t find it in the supermarkets. Quarg’s only American appearance was in one of the projects at the NASA Space Center in Houston where quarg was included in the daily breakfast of astronauts.Further possibilities in quarg research and development are various drying process and direct acidification technique. The food service industry will
undoubtedly also work with quarg and incorporate it into the world wide spectrum of Indian cuisine.

**Figure 4.1:** Flow Diagram for manufacture of Fiber and phytosterol enriched quarg cheese
Introduction

Cheese, the nature's wonder food and the classical product of biotechnology, is a highly nutritious food with good keeping quality, enriched pre-digested protein with fat, calcium, phosphorus, riboflavin and other vitamins, available in a concentrated form. It has been reported to have therapeutic, anticholesterolemic, anticarcinogenic and anticariogenic properties beyond their basic nutritive value. Scenario of cheese production in India is quite right because of the facts that cheese has all the beneficial attributes of an ideal dairy product and the emergence of new global economic reforms based on globalization and liberalization in the marketing arena that has unfastened the door to the Indian dairy industry to penetrate the international cheese market. Cheese is appreciated by consumers for the great interest and variety it adds to the eating experience. It has an excellent image, being perceived as healthy, natural and nutritious. Cheese has, therefore, been truly classified as a value added product and is consumed in various other forms like dietetic foods, snacks fast foods and spreads. With the triumphant achievement in the arena of dairy science and biotechnology since the last two decades, lot of advancement have been made in cheese technology to provide ease in its processing and to gift the mankind with novel kind of cheese with improved flavour and textural characteristics.

Whey Cheeses are solid, semi-solid, or soft products which are principally obtained through either of the following processes:

- the concentration of whey and the moulding of the concentrated product;
- the coagulation of whey by heat with or without the addition of acid.

In each case, the whey may be pre-concentrated prior to the further concentration of whey or coagulation of the whey proteins. The process may also include the addition of milk, cream, or other raw materials of milk origin before or after concentration or coagulation. The ratio of whey protein to casein in the product obtained through the coagulation of whey shall be distinctly higher than that of milk. The product obtained through the coagulation of whey may either be ripened or unripened. Whey Cheese obtained through the concentration of whey is produced by heat evaporation of whey, or a mixture of whey and milk, cream, or other raw materials of milk origin, to a concentration enabling the final cheese to obtain a stable shape. Due to their relatively high lactose content these cheeses are typically yellowish to brown in color and possess a sweet, cooked, or caramelized flavor. Whey Cheese obtained through the coagulation of whey is produced by heat precipitation of whey,
Whey cheeses are cheese-like products. These are very popular in Norway, Greece, and Italy. Whey cheeses like Gjetost, Mysost, and Gudbrandsdulsost are produced in Norway, while Manouri, Anthotryos, Cryzittroa, and Giza in Greece. The names of whey cheeses may refer to a mixture of whey and milk or cream, with or without the addition of acid. These whey cheeses have relatively low lactose content and a white to yellowish color.

Whey, a protein complex derived from milk, is being touted as a functional food with a number of health benefits. The biological components of whey, including lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glycomacropeptide, and immunoglobulins, demonstrate a range of immune-enhancing properties. In addition, whey has the ability to act as an antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial, and chelating agent. The primary mechanism by which whey is thought to exert its effects is by intracellular conversion of the amino acid cysteine to glutathione, a potent intracellular antioxidant. A number of clinical trials have successfully been performed using whey in the treatment of cancer, HIV, hepatitis B, cardiovascular disease, osteoporosis, and as an antimicrobial agent. Whey protein has also exhibited benefit in the arena of exercise performance and enhancement.

In recent years, milk constituents have become recognized as functional foods, suggesting their use has a direct and measurable effect on health outcomes. Whey, a by-product of cheese and curd manufacturing, was once considered a waste product. The discovery of whey as a functional food with nutritional applications elevated whey to a co-product in the manufacturing of cheese. Milk contains two primary sources of protein, the caseins and whey. After processing occurs, the caseins are the proteins responsible for making curds, while whey remains in an aqueous environment. The components of whey include beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin, lactoferrin, immunoglobulins, lactoperoxidase enzymes, glycomacropeptides, lactose, and minerals. In addition, whey derived from buttermilk versus cheese contains the lipid sphingomyelin.

Today, whey is a popular dietary protein supplement purported to provide antimicrobial activity, immune modulation, improved muscle strength and body composition, and to prevent cardiovascular disease and osteoporosis. Advances in processing technology, including ultrafiltration, microfiltration, reverse osmosis, and ion-exchange have resulted in development of several different finished whey products. Whey protein concentrates (ranging from 80-95 percent protein), reduced lactose whey, whey protein isolate, demineralized whey, and hydrolyzed whey are now available commercially. Each whey product varies in the amount of protein, carbohydrates, immunoglobulins, lactose, minerals, and fat in the finished product. These variables are important factors in the selection of whey fractions for specific nutritional applications.

**Whey cheese types**

Whey cheeses are cheese-like products. These are very popular in Norway, Greece, and Italy. Whey cheeses like Gjetost, Mysost, and Gudbrandsdulsost are produced in Norway, while Manouri, Anthotryos, Cryzittroa, and Giza in Greece. The names of
whey cheeses in Greece indicate their quality. For example, Manouri contains 30% fat and 65% TS while the fat content of Antotryos is only 19.25%. Ricotta cheese is another cheese which is popular in Italy and in many other countries. Whey cheeses are also known by different names in different countries. Some of the popular names are, Schoftenzieger, Schabzieger (Germany); Mascarpone (Switzerland); Klila (Tunisia); Nicotta (North Africa); Zieger (Romania); Kaukaz (USSR); Anan (Cyprus); Lour (Iraq); Ricotta Fresca (Brazil); Urda (Israel and Czechoslovakia); Zinicica (Czechoslovakia) and Karicha (Lebanon).

**Principle of cheese making from whey**

The basic principle involved in making most of the whey cheeses involves coagulation and subsequent separation of whey proteins by heating of whey which may also be supplemented with milk or milk fat. Whey cheeses may be classified as Brown whey cheese and re-cooked whey cheese. The classification of Brown whey cheese as cheese is somewhat misleading as they contain all the whey constituents including lactose which otherwise is eliminated in cheese. From the standpoint of alleviating disposal problem of cheese whey Brown cheeses offer a much better alternative as they utilize all the whey solids in comparison to re-cooked cheeses which result in a significant amount of partially de-proteinised whey. In a cheese plant, two types of whey, i.e., sweet and acid whey are produced as a by-product. Sweet whey results from the production of ripened cheeses like Cheddar, Swiss, Blue etc. and is recommended for most of the whey cheeses. Acid whey obtained from soft cheeses such as cottage, cream, paneer etc. can be used for cheese making after neutralization but some of the cheese quality is lost. Acid whey below a pH 6.0 results in a coarse texture, reddish colour and sour flavour of cheese. Different methods by which whey can be used in cheese making are as follows:

- Heating of whey with or without acidifying and separation of proteins and fat as curd which after drainage gives a cheese.
- Heating of whey, separation of coagulated whey proteins and its addition to cheese milk to increase yield. Whey proteins must be denatured before addition to cheese milk. Addition of acid whey, or acid whey powder and/or lactic, citric or acetic acid improves precipitation of whey proteins. Whey protein concentrates should be added to cheese milk in liquid form and the powdered preparations dissolved and heat treated prior to use to minimise bitterness in cheddar cheese.
- Heating of whey for obtaining curd with denatured proteins which are added in different proportions to processed cheese blend or are converted into soft cheese like products.
- Condensation of whey by heating or by reverse osmosis for making Brown whey cheese.

Modifications in the method of heating include heating of whey with a large number of fine tubes inside the vat or by making special arrangements in the cheese vat that permits the use of hot serum for pre-heating the whey. Considerable saving in
temperature. Reverse osmosis and addition of dry butter milk to liquid unprocessed whey have been used to develop hard as well as spreadable Mysost like products. Lactose content of buttermilk containing spreads can be reduced by using lactose reduced buttermilk powder produced by ultrafiltration process (UF). Hydrolysis of lactose in condensed rennet whey using Maxilact enzyme helps in controlling sandiness and labour could be achieved by using a screen to drain the mixture of whey and curd or by providing a mechanical system for collection and transfer of curd into moulds for subsequent drainage.

**Manufacture of whey cheeses**

Norwegian Brown whey cheeses are made from goat's milk, cow's milk or a combination of both. Gjetost is made only from goat's milk while cow's milk is used for Mysost. Addition of cream to Gjetost yields a quality cheese known as Primost which has a light tan colour and smooth and creamy body texture. Gjetost is darker Brown in colour with coarser texture. The flavour is similar to cream caramel. A mixture of 88% goat's milk and 12% cows' milk is used for another Brown cheese known as Gudbrandsdulsost. The unit operations involved in the manufacture of Brown cheeses include evaporation, rapid cooling with vigorous stirring, packaging and solidification. The whey (sweet or acid) after filtration is centrifuged and pasteurised. Milk and cream to the extent of 35 to 40% are added to increase the protein and fat content in the cheese. Mixture is then concentrated to 50 to 55% TS in conventional vacuum evaporator. Second stage concentration to 80-84% TS is carried out in a kettle under reduced vacuum pressure. The vacuum is thereafter released and temperature is raised to allow the concentrate to develop the desired brown colour and flavour. The concentrate is then filled into containers where it is cooled with stirring. In a modified method, the concentrate from the evaporator is further concentrated to 75 to 82% TS in a specially designed kettle (Gryta) at a temperature of 95-96°C and then transferred to scraped heat exchangers for kneading and rapid cooling to 75°C or lower. Vigorous stirring in both the methods is important to prevent formation of lactose crystals aggregates and the sandiness defect. Finally the viscous mass is packed in Al-foils, plastic bags or cups and cooled overnight so that it solidifies. It is now a standard practice to add 10 mg ferrous sulphate per 100 g cheese to increase the iron content.

Greek whey cheeses are manufactured in a similar way from sheep's milk but other types of whey can also be used. The manufacturing steps include filtration of sweet whey (pH above 6.0) followed by heating to 90°C for 40 to 45 min. Cream and milk (optional) are added at 40°C and salt @ 1.0-1.5% at 75°C. The curd after coagulation is cooked at 88-92°C for 15-30 min and then transferred to moulds and drained. Manouri and Myzithra that are to be used fresh are heated at lower temperatures. When the products are to be dehydrated afterwards, heating is done to a higher temperature.

Reverse osmosis and addition of dry butter milk to liquid unprocessed whey have been used to develop hard as well as spreadable Mysost like products. Lactose content of buttermilk containing spreads can be reduced by using lactose reduced buttermilk powder produced by ultrafiltration process (UF). Hydrolysis of lactose in condensed rennet whey using Maxilact enzyme helps in controlling sandiness and
also in the production of optimally caramelized whey cheeses. A new technology involves admixing of highly ultrafiltered whey with cream followed by packaging of the mix and heating at 90°C for 50 to 90 min. Denaturation of proteins occurs in the packages and the cheese is formed. UF retentate from cottage cheese whey mixed with butterfat without any additional source of milk solids has also been used.

The best known whey cheese on universal scale is Ricotta cheese which is also known as albumin quarg. It is known as Ricotone in certain parts of U.S.A. if made only from whey. The traditional Italian style Ricotta cheese is primarily a heat coagulated whey protein. However, in some countries, modern Ricotta (Re-cooked cheese) is made from milk-whey or buttermilk-whey mixture or even from whole milk by heating the mix to 80° to 90°C followed by acidification to a pH value below 5.9. The coagulated curd is separated and filled in perforated metal cans for draining. Salt and lactic starter may be added after the curd is cooled to 30°C. The drained curd is ready for consumption. In one of the mechanised methods separation of coagulum from the surface of the heating vat is accomplished by a series of peddles which transfers the curd into perforated conveyor. However, this device is suitable for Ricotta cheese produced from whey-milk mixture in which the high casein content provides cohesiveness to the otherwise fragile whey protein coagulum. In a separate development, heating vat has been replaced by a tubular holding section which uses whey after concentration of whey protein to about 2% by UF. A fully automated line which combines the heating, drainage and filling of moulds has also been developed for Ricotta cheese.

A method for the manufacture of whey cheese from buffalo milk whey known as Requeson has been developed by using calcium chloride or citric acid and subsequent heat precipitation of whey proteins. Another whey cheese from a mixture of whey and cream is manufactured by concentrating the whey to 10-11% TS by reverse osmosis process followed by addition of cream. The mix is further concentrated in two steps to the desired level under vacuum.

**Composition of whey cheeses**

Whey cheeses differ in composition mainly according to fat and moisture content. The differences in technology, composition of whey, addition of whole milk and type of milk, influence the composition of cheese. Other factors which affect the quality of whey cheese include quality of whey, method and degree of heating the whey, whey acidity and addition of calcium chloride and sodium chloride. The compositional data is presented in the Table-1. The amount of fat in Ricotta cheese is determined by the type of cheese from which the raw material i.e. whey was obtained, and by the amount of milk fat contributed through any added milk.
Table 1. Chemical Composition of Whey cheese varieties

<table>
<thead>
<tr>
<th>Whey Cheese</th>
<th>% Chemical Composition</th>
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<tbody>
<tr>
<td></td>
<td>Fat</td>
</tr>
<tr>
<td>Mysost</td>
<td>28-30</td>
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<tr>
<td>Manouri</td>
<td>36.5</td>
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<tr>
<td>Myzithra</td>
<td>16.0</td>
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<tr>
<td>Dry Myzithra</td>
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<tr>
<td>Ricotta</td>
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<tr>
<td>Ricotta from can’s sheep's</td>
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<tr>
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<td>22.4</td>
</tr>
<tr>
<td>Ricotta from dry Ricotta</td>
<td>2.5</td>
</tr>
<tr>
<td>Dry Ricotta</td>
<td>5</td>
</tr>
</tbody>
</table>

Nutritive value of whey cheeses

Whey cheeses are distinguished in that they have different kind of proteins than the other cheeses. Whey proteins are rich in the amino acids, methionine and cystine and their essential amino acids content is more than sufficient to cover the needs of man as recommended by FAO. The biological value of whey proteins is 1.0 against 0.8 of casein and 0.9 of coprecipitates of casein and whey proteins. When cheese is made by heating whey there is no appreciable reduction in biological value of proteins. The biological value of cheese proteins is 0.91 as against 0.92 of fresh whey, 0.91 of protein powder of UF whey and 0.94 of lactalbumin. Also, the cheese was not inferior in available lysine against other products and it had true digestibility 0.97. The diet of children could be supplemented with whey cheese.

Norwegian whey cheeses are quick source of energy (18,000 kJ/kg) as they contain high amount of lactose. The spreadable product, however, has lower energy content. The high moisture content of Ricotta cheese reduces its energy value and, therefore, it is known as slimming food. It is a healthful food because of high protein content of good quality.

Shelf-life of whey cheeses

The high lactose content of Mysost types of cheeses is the primary reason for their excellent shelf-life and microbiological stability. The whey cheeses produced in Greece in salted form (2.5%) and with the addition of starter (1%) have a shelf life of 6 months. Ricotta cheese is very susceptible to rapid spoilage by moulds, yeast and bacteria because of its high moisture content, high pH and the contamination to which the surface is exposed. However, pH adjustment and filling under heat application improve the keeping quality of the product. Fresh Ricotta packaged in plastic bags has a shelf life of 2-3 weeks. Packaging in an atmosphere of nitrogen or carbon dioxide increases its storegability to 6 weeks. High moisture (50-80%) whey cheeses are, therefore, usually consumed fresh. The cheeses are marketed one day after their manufacture and disposed within a week.
Uses of whey cheeses

Whey cheeses are traditional in several countries. Norwegians commonly consume brown whey cheeses in sandwiches or Scandinavian-type crispbread. Brown whey cheese has a sweet taste. Manouri and Anthotyros are consumed as table cheeses in Greece. Unsalted cheeses are eaten with honey. Myzithra is sometimes used as a table cheese but more often in preparations of certain foods and cheese pies. Fresh Ricotta cheese is white, moist and grainy. Its appearance resembles cottage cheese curd but the consistency is very dry and crumbly. It has a bland to semi-sweet flavour. Fresh Ricotta is ideal for cooking purposes as it does not develop lumps, and forms a smooth emulsion with fat. The potential uses of fresh Ricotta cheese are in combination with fruits or honey for breakfast dishes, in creamy desserts, cake fillings, baked products, Italian pasta products and Indian sweets. Dried Ricotta is suitable for grating and as a compliment for other cheeses for more pronounced flavour. Processed cheese is also produced (pH 5.9, 23% FDM) from Ricotta and Cheddar cheese.

Conclusion

Whey, a protein complex derived from milk, is being touted as a functional food with a number of health benefits. The biological components of whey, including lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glycomacropéptide, and immunoglobulins, demonstrate a range of immune-enhancing properties. In addition, whey has the ability to act as an antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial, and chelating agent. Several types of whey cheeses are being manufactured commercially and consumed throughout the world. These cheeses are nutritionally sound and enjoyed for its functional attributes.

References

TECHNOLOGY OF FRUIT FLAVORED AND FIBER FORTIFIED FERMENTED DAIRY PRODUCTS

S.K. Kanawjia, P. N. Raju, Yogesh Khetra and Alok Chatterjee

Introduction

India has a vast horticulture base with a wide range of fruit varieties that led to one of the largest fruit producing countries in the world. The production of fruits in India is confined to 27.8 MT, which amounts to 8.1% of the total world fruit production. Post harvest losses of semi-perishables and perishables in India are also amongst highest in the world at 30-35 % resulting in a great loss to our economy (Kachru, 2006). The major hurdle in the success of fruit-based agro process industry is the low 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 fruits in an effective manner. Keeping in view, the market trend in western dairy market, incorporation of fruits in to fermented milk products 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. Recently, there has been an increased trend to fortify cultured milk products with fruit juices/pulps. 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 products like fruit-yogurt beverages. Addition of fruit preparations, fruit flavors, and fruit purees not only enhances versatility of flavor, texture, color, variety to fermented milks but also contributes to significant amount of dietary fiber with an additional healthy image tag.

Health benefits of fruits

Keeping in view the market trends, incorporation of fruits in traditional fermented milk products not only aids in value addition and product diversification but also helps in checking the post harvest losses and hence economic loss. It may also enhance the profitability of milk and fruit producers as well as processors. Fruits are rich sources of various important phytonutrients namely, vitamins, minerals, antioxidants and dietary fibers. Various researchers have described the effect of fruit addition on mineral contents of yogurt. A number of scientific studies have been carried out to prove the beneficial effects of fruits in human health. Current evidences collectively demonstrate that fruit and vegetable intake is associated with
improved health, reduced risk of various types of cancers, CVD, hypertension and possibly delayed onset of age related indicators.

Milk-fruit based products

In the formulation of milk-fruit based fermented products, processed fruits are widely employed and they may be added to cultured milk in various forms namely fruit purees, fruit pieces, fruit syrup/juices, crushed fruit, frozen/osmodehydrofrozen fruits, fruit preserves and other miscellaneous fruit products. Researchers have outlined processes for making fruit yoghurt with fruit concentration mainly ranging from 4-20%. Addition of mango pulp more then 4.0 per cent was reported to adversely affect delicate yoghurt flavor and also the body and texture irrespective of homogenization pressure. However, increased pressure up to 200 bars may help in reducing whey separation and provide smoother consistency. Suitability of different fruits i.e. mango, sapota, papaya, pineapple, kokum @ 10, 15, 20% levels each was studied for preparation of fruit yoghurt and it was concluded that that mango pulp and pineapple juice could be used satisfactorily up to 20% level. However, sapota pulp, papaya pulp and kokum juice produced inferior quality yoghurt. Fruit dahi was prepared using mango, banana, pineapple and strawberry @ 6, 8, 6 and 4 percent levels each and mango fortified dahi was found to be most acceptable on basis of organoleptic quality. Fruit based shrikhand has also been prepared wherein fruits like apple, papaya, mango were employed. Coconuts have also been employed in yoghurt production wherein four types of yoghurts were made from mixtures of cow milk and coconut milk in different combinations. Using coconut milk in yoghurt production could be an interesting alternative option in the regions with high coconut production. Sterile extracts of Phaseolus vulgaris (caraota) and Vigna sinensis (frijol), as partial substitutes (which replaced milk: 10, 20 and 30%) have been used to develop novel probiotic drink with increased levels of protein, soluble and insoluble fiber, available and resistant starches and 81% protein digestibility. Soursops (Annona muricata L.) that are highly aromatic fruits with white juicy flesh native to tropical North and South America were incorporated in yoghurt @ 10 and 15 %. These yoghurts were reported to provide high percentage daily values of zinc, phosphorus and calcium and a good level of protein. Fruit (Arecaceae), known as Acai in the Brazilian Amazon region, is dark purple with a high anthocyanin and phenolic content. The novel natural colorants from E. oleracea juice could be considered as “functional” ingredients for their anti-oxidant and anti-radical activity. The protein profile of the E. oleracea (10%, w/w) containing yogurt was essentially identical to the untreated control yogurt.

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.1). 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.

**Preparation of mix**

↓

Heating (60°C)

↓

Homogenization (2000psi (I stage) – 500psi (II stage))

↓

Heating (85°C/15min)

↓

Cooling (32±1°C)

↓

Sugar Syrup (pasteurized) ← Inoculation (1.5 %)

↓

Fruit pulps (pasteurized)

↓

Incubation (32°C)

↓

Cooling and storage (6±1 °C)

**Fig.1. Schematic Diagram for Fruit Dahi Manufacture**

**Fruit Lassi**

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. Cultured dairy products are an excellent
medium to generate an array of products that fit into the current consumer demand for health-driven foods. Development of mango based *lassi* like beverage would not only provide enhanced nutrition, value addition and product diversification but also help in curtailing post harvest losses in mango. If the shelf life of mango *lassi* could be extended, especially by the use of biopreservatives, it would offer stiff competition to expensive soft drinks in the beverage market and thus enhancing the profitability of milk and fruit producers as well as processors. The optimization of ingredients for the formulation of mango *lassi* was carried out using Response Surface Methodology with Central Composite Rotatable Design (3 variables and 5 levels each). A total of 20 trials were conducted and the individual and interactive effects of milk fat (0.48 - 5.52 %), sugar (12-17 %) and mango pulp (3.95-12 %) on sensory and physicochemical properties of mango *lassi* were studied. The most acceptable mango *lassi* formulation was finally selected by maximization of all the sensory responses. A good quality, highly stable mango *lassi* with less than 1% whey separation was thus obtained using EPS (exopolysaccharide) producing cultures in combination with pectin as stabilizers. The shelf life of mango *lassi* was further extended up to 50 days using Microgard as biopreservative. Technologies have also been developed to manufacture lassi utilizing certain other fruits such as banana, straw berry, pineapple, etc.

**Dietary fiber**

Dietary fiber consists of the remnants of edible plants and associated substances, polysaccharides and carbohydrate analogues, and lignin, which are resistant to digestion and absorption in the small intestine, and is partly fermented by bacteria in the large intestine to form various metabolic products. Many fiber preparations also provide bioactive plant photochemical. Vegetables and cereals are natural sources of fiber, with whole grain bread being a particularly good source. In most countries the recommendation for dietary fiber intake is 25 to 35 g/day. Dietary fiber helps to keep the digestive system functioning effectively, and it assists in balancing blood glucose levels and weight control. Fiber may even help to reduce the risk of type 2 diabetes, coronary diseases and certain cancers. Milk and milk products, with no native dietary fiber in them, form an essentially low-residue diet. The fortification of milk products with dietary fiber as a physiologically functional principle would help enhance the overall health value of these highly nutritious but sometimes ‘suspected’ or ‘risk-raising’ commodities. In recent times the concept of fortification and enrichment of foods which are deficient in certain nutrients or ‘health factors’ has been extended to health foods.

**Health attributes of fibers**

It was in 1974 when Burkitt, Painter and Trowell observed that the rural Africans did not have many of the diseases that plagued the West, and they theorized the positive effects of the high-fiber diet on the gut. Thus, fiber was launched from
merely being roughage to a substance with many possible therapeutic and preventive roles in several gastrointestinal disorders including enteritis. Dietary fiber has been found to reduce the risk of hyperlipidemia, heart disease, diabetes mellitus and obesity in susceptible genotypes (DeVries, 2003). The best accepted beneficial effect of dietary fiber is relief of constipation. Fiber supplements such as psyllium-seed fiber and methylcellulose, or food components such as wheat bran are known to be used in the treatment of chronic constipation. Several epidemiological studies have indicated a strong link between a high-fiber diet and the prevention of coronary heart disease. Viscous fibers such as locust bean gum, konjac mannan, psyllium and legume fibers (Jenkins, Wolever, and Rao, 1993), and also pectin, guar gum and high-fiber food fraction such as oat bran and oatmeal (Bartnikowska, 1999), all providing 12 to 30g fiber daily, have been shown to reduce total and LDL-cholesterol levels by 10 to 20 per cent with a less fall in HDL-cholesterol levels. Further, fiber-rich meals help in weight management. High-fiber diets may also provide benefits to diabetic patients by lowering blood glucose concentration, reducing postprandial insulin levels and antidiabetic drug requirements and decrease blood lipid concentration. Fiber has been associated with the prevention of cancer. On the basis of the several health promoting roles of dietary fiber, WHO recommended an intake of 27-40 g dietary fiber per day (Cho, Sullivan and Rickard, 1999) and Indian Council of Medical Research (ICMR) and National Institute of Nutrition (NIN, Hyderabad) recommend 40 g dietary fiber per day for Indians.

Commercial fiber preparations

Several fiber preparations, both soluble and insoluble types, are offered to the food industry for commercial applications in North America and Europe, and a range of food products are fortified with such ingredients. Among soluble dietary fiber, several non-digestible oligosaccharides are available in international markets under various trade-names (Roberfroid, 1999). These include natural fructooligosaccharides e.g. inulin (Raftline®) and its hydrolysis product oligofructose (Raftilose®), synthetic fructooligosaccharide like galactooligosaccharide (Oligomate®), neosugar (Neo-sugar®, Actilight®), transgalactooligosaccharides (Cup-oligo®), isomaltooligosaccharides (Isomalto®) and palatinose condensates which includes polydextrose (Polydextrose®), pyrodextrins, sololigosaccharides (Soya-oligo®) and xylooligosaccharides (Xylooligo®).

Dietary fiber fortified dairy products

Fortification with natural fiber-rich foods

Fiber-rich food ingredients provide pectin, hemicellulose, cellulose and lignin in the dairy foods, besides, of course, contributing valuable micronutrients. There are a few dairy products conventionally manufactured include fruit-flavoured yoghurt, ice cream, ‘milk-shakes’, fruit-and-nut ice cream is most popular. New dairy foods
developed recently include vegetable yoghurts like sweet potato yoghurt and yam yoghurt, vegetable ice cream e.g. spinach flavoured one, ice cream made with beets, celery and carrots, oatmeal and prune ice cream and certain desserts. There are quite a few traditional milk sweets that contain substantial quantities of fiber e.g., Gajar-pak (carrot halwa), Ghiya-ka-halwa (bottle-gourd halwa) (Aneja, et al., 2002), Doda barfi, and Kaju-burfi. Traditionally made cereals-based milk desserts like kheer, Dahi, yoghurt and Raabdi are some dairy food sources of dietary fiber in Indian diets. In the United States and Europe, yoghurts carrying whole cereal grains (e.g. wheat and oat), soy fiber and fruits like cranberries, blackberries, raisins, blueberries, walnuts, hazelnuts, etc. are already to be found on the market under various names such as Yoplait Breakfast Yoghurt, Yoghurt Diet Meal, Fruits of the Forest and so on. These foods may also be the natural sources of some bioactive components like carotenoids, antioxidant vitamin C and E, saponins, sterols, phenols, allium compounds and indoles. Most of them have been largely associated with anti-tumor activity and hypolipidemic properties.

**Fortification with commercial fiber preparations**

There are several insoluble preparations like purified cellulose, microcrystalline cellulose, methyl cellulose, lignin, wheat fiber, oat fiber, apple fiber, orange fiber, pea fiber, etc. as also are soluble fiber preparations such as inulin, short chain fructooligosaccharides, β-glucan, psyllium, gums and mucilages which are available commercially. The gums available in the market may be of various types viz., seed gums (locust bean gum, guar gum, psyllium), plant exudates (gum arabic, gum ghatti, gum karaya, and gum tragacanth) and microbiologically produced gums (xanthan gum and gellan gum) to be used as the concentrated source of dietary fiber. Soluble fiber such as Inulin and short-chain fructooligosaccharides, also serve as prebiotics and enhances human gastrointestinal system and immune system (Ohr, 2004). These have found successful use in yoghurt. In Japan, several companies are using various oligosaccharides to fortify infant milk formulas, baby foods and yoghurt e.g., Yakult Honsha co., Suntory Co. and Morinaga Food Industry. In Belgium, a dietary fiber-fortified fermented milk drink called Fyos containing inulin is very popular. The Ultra Slim Fast, a beverage from RTS Deans Foods, prepared by the fortification of skim milk with purified cellulose, corn bran, carrageenan and guar gum has been reported to supply 5g dietary fiber per 12-ounce (340g) serving. The fiber preparations from Roxler International's Bakeflora line is in use to replace sugar 1:1 to manufacture low-carbohydrate, low-energy, dietary fiber-enriched formulations, GTC Nutrition's Natureal GI oat bran concentrate and Matsutani America's Fibersol-2 resistant maltodextrin are claimed to be ultra-low glycemic fibers with a high potential for food fortification. More recently NDRI developed fibre rich paneer, and Quarg cheese using inulin, soy and oat fiber.
Conclusion

Nutrient-rich and energy-rich milk products are often associated with potential risks of developing certain health problems. Fruits Dietary fibers have come to be widely recognized as a health-promoting food constituent, but is absent in conventional dairy products with a few exceptions which contain non-dairy ingredients. Fruits and Fiber incorporation in protein- and/or fat-rich dairy products can potentially reduce the health risks associated with these products. Incorporation of fiber-rich natural food ingredients such as fruits and vegetables wherever possible is apparently the most practical way of enhancing the functionality of dairy food as, in fact, has already been practiced in products such as dahi, lassi, yoghurt, ice cream, paneer, cheese and certain traditional milk sweets.

References

Introduction

Cottage cheese, designed as slim cheese with low calorific value (96 Kcal/100g), and low fat with reduced cholesterol content is very much suitable for the people suffering from the metabolic and physical mayhems like lactose intolerance, atherosclerosis, obesity etc. So its incorporation as ethnic food in the diet list of modern consumers rummaging around newer taste everyday may wake up the dormant opportunity to the Indian dairy industry to brace our national economy, which requires extensive studies to make it accuser to the consumers in terms of quality and palatability as well as to the industry in terms of technological accessibility.

Cottage cheese has a pleasant mild flavour which is attuned to the olfaction of Indian people with its widespread consumer appeal both as a savoury and dessert product, and its potential as low cost, good quality high protein and low fat product, the consumption of Cottage cheese seems to increase significantly, as because of the consciousness of a large group of Indian population concerning over-weight and cardiovascular as well as other metabolic ailments. This is also the main reason for a drastic boost in production of Cottage cheese in the USA and many other countries. Popularising Cottage in India will not only help to increase the nutritional status and provides a substitute for ripened varieties of cheese, a luxurious item on account of its high cost but also satisfy the crave of modern patrons for tasting cheese, who have grown phobia over obesity and cardiovascular as well as metabolic disorders.

Nutritional attributes

There is a bright future for production of Cottage cheese production in our country due to its good nutritional profile. Like other developed countries in India also, a large number of people have grown phobia over obesity in India also Modern human nutrition nutritionists suggest not to consume foods with high calorific value and also modern nutrition demands that the diet be varied enough in respect of their food values. In this respect, Cottage cheese having fat not more than 4 per cent of the final product would be a suitable alternative to the other popular dairy products. Table 2 shows the comparative calorific value of some popular dairy products. A half-cup portion of Cottage cheese supplies about as much protein as two ounces of cooked, lean meat, poultry or fish (14 g). A half-cup of 2 per cent low fat Cottage cheese contains 77 mg of calcium with only 102 calories (IDFA, 2000). Nutritionally, cottage cheese is a wholesome low-calorie food, however, it contains lower levels of calcium of about 30 mg/100g for dry curd, 60 mg/100 g for creamed curd and 68...
mg/100 g for low fat creamed curd as compared to rennet coagulated cheese having calcium content in the range 700-950 mg/100 g (Guinee et al., 1993). Wong et al. (1976) suggested that approximately 50 per cent of the calcium in Cottage cheese comes from cream dressing. Addition of CaCl₂ to milk has been observed not to affect the calcium content of Cottage cheese; however, calcium level may be raised by the addition of calcium salts (Chloride, lactate or phosphate) on to cream dressing without any detrimental effects on sensory and microbiological quality (Shelef and Ryon, 1988).

<table>
<thead>
<tr>
<th>Foods</th>
<th>Energy (kcal/100 g.)</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>Soft cheese</td>
<td>285</td>
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<tr>
<td>Yoghurt</td>
<td>180</td>
</tr>
<tr>
<td>Cottage cheese</td>
<td>96</td>
</tr>
</tbody>
</table>


Cottage cheese contains about 4 mg sodium per g, most of which is added with the cream dressing since only about 3 per cent of the sodium in milk (~ 0.5 mg/g) is retained in Cottage cheese curd (~ 0.11 mg/g) after three washings (Wong et al., 1976; Bruhn and Franke, 1988 & Demott et al., 1984). Attempts have been made to manufacture low sodium Cottage cheese with sensory qualities analogous to regular Cottage cheese by reducing the sodium content of the dressing by 25 per cent or by replacing NaCl upto 50 per cent with KCl (Demott et al., 1984 & Wyatt, 1983).

**Probiotic cottage cheese**

The development of technology of Probiotic Cottage cheese developed is intended to amalgamate some health benefits of probiotic culture to the product with special emphasis on the hypocholesterolemic effect of probiotic to reduce the risks of atherosclerotic CVDs. Animal study using rat model revealed that feeding probiotic cheese considerably reduces plasma cholesterol and plasma LDL levels.

**Flavour**

Cottage cheese is mildly acidic in taste with the delicate aroma of diacetyl. The acidic taste of Cottage cheese is largely due to lactic acid, which is present at concentrations ranging from 124 to 452 mg/kg. The other acids predominantly present in Cottage cheese are formic acid (23-306 mg/kg), acetic acid (11-292mg/kg) and low concentrations (<1 mg/g) of propionic and butyric acids (Brocklehurst and Lund, 1985). Formic, acetic, propionic and butyric acids are volatile and hence, contribute to the aroma of Cottage cheese. The most idiosyncratic flavour compound in Cottage cheese is diacetyl, produced by oxidative decarboxylation of α-acetolactic acid. The development of diacetyl largely depends upon pH and generally occurs at pH values more than 5.5 (Collins, 1972). In Cottage cheese, the acceptable levels of diacetyl are estimated to be approximately 2 ppm.
and a diacetyl/acetaldehyde ratio of 3 to 5 is desirable for pleasant and good flavour (Lindsay et al., 1965 & Hempenius et al., 1965).

**Keeping quality and preservation**

Cottage cheese is highly perishable product and suffers from the limited shelf life due to high moisture content. Short shelf life is one of the most serious problems in marketing and distribution of Cottage cheese to the restaurants and fast food centres, which consume the product in large quantity a lot as base material for patties, sandwiches etc. Shelf life of a creamed Cottage cheese is the period during which the cheese suffers no marked deterioration in quality at storage temperature of approximately 7.2°C (Kosikowski, 1982). For most commercial Cottage cheese, the shelf life does not exceed 7 days, while some, where strict quality control is practiced, may exhibit a shelf life of 15 days (Luck et al., 1977 & Johnson, 1979). The loss of flavour quality in Cottage cheese results primarily from the growth of undesirable microorganisms. The spoilage of Cottage cheese is mainly because of the growth of undesirable microorganisms’ microbial growth, which brings about several physico-chemical changes leading to the development of off-flavour and discoloration of the surface. Psychrophilic growth results in the loss of diacetyl and in the development of “fruity fermented”, “bitter”, “rancid” and “putrid” flavour and a slimy curd. Yeast and mold growth results in flavours described as “yeasty”, “moldy”, “stale”, and “fruity” and discoloration of the product (Elliker, 1954).

Cottage cheese is highly perishable product and suffers from the limited shelf life due to high moisture content. Short shelf life is one of the most serious problems in marketing and distribution of Cottage cheese to the restaurants and fast food centres, which consume the product a lot as base material for patties, sandwiches etc. The spoilage of Cottage cheese is mainly because of microbial growth, which brings about several physico-chemical changes leading to the development of off-flavour and discoloration of the surface.

Since past few decades, several food scientists for the preservation of this product have investigated a number of chemical and physical treatments for the preservation of this product. This section will review the literature to delineate various works perspicuously on the preservation of Cottage cheese with lucid emphasis on the natural preservation.

**Antifungal agent**

Apart from some conventional approaches like regulation of pH of creamed cottage cheese application of variety of antifungal agents has been attempted in controlling the spoilage of Cottage cheese for its preservation.

**Sorbic acid and potassium sorbate:** The shelf life of cottage cheese can be greatly extended (>~75%) by adding sorbic acid or potassium sorbate (0.75 %w/w), which inhibit psychrotrrophic bacteria and molds without producing objectionable flavours.
which prolonged the shelf life of the product to around 40 days. Dehydration:

For the US Army, a high quality dehydrated cottage cheese with 1.5 per cent moisture had been developed from frozen creamed and salted curds. The product is freeze dried to give a highly soluble powder suitable for reconstitution, and produces high quality fresh product upon the addition of clean water (Kosikowski, 1982). The frozen creamed curds after freeze drying under vacuum without thawing was stored under nitrogen in sealed tin cans with less than 2.0 per cent oxygen in the headspace gas. The shelf life of such dehydrated Cottage cheese was about 1 year at 22°C and 2 years at 5°C.

Morley (1983) also reported the prolonging shelf life of Cottage cheese by adding sorbic acid and acidification of the dressing mixture. The keeping quality of Cottage cheese treated with 0.075 per cent sorbic acid is reported to be almost double than that of untreated samples. However, a slight after taste or bitter taste is occasionally detected when 0.1 per cent potassium sorbate is added (Bodyfelt, 1979).

Buffalo milk Cottage cheese with 0.1 per cent added sorbic acid remained well for 2 weeks when packed in containers treated with 1 per cent sorbic acid (Mohan et al., 1982). They found that sorbic acid was more effective over sodium propionate at 0.05 or 0.10 per cent levels to improve shelf life and acceptability of creamed Cottage cheese.

Monolaurin: Studies with monolaurin, a naturally occurring monoglyceride, which can be legally added to Cottage cheese, indicated that the incorporation of upto 500 ppm monolaurin in Cottage cheese can result in the inhibition of 90 per cent yeast by upto 5 days at 6°C without any adverse effect on organoleptic properties (Bautista et al., 1992). The monoglyceride ‘monolaurin’ has been shown to possess anti-microbial properties as well as being an emulsifying agent. Incorporation of monolaurin into naturally contaminated Cottage cheese at levels of 250 and 500 ppm resulted in greater than 90 per cent inhibition of both Pseudomonas spp. and coliforms during 7 days of storage at 6, 15 and 21°C. There was also greater than 90 per cent inhibition of growth of yeast and fungi under the same storage conditions in the presence of monolaurin.

Physical treatments

Sterilization: Lexag (1978) patented a method for extending the shelf life of cottage cheese by adding a stabilizer and then sterilizing in sealed packages. The stabilizers included potato starch, vegetable protein, gelatin, pectin, citric acid, phosphate, citrate and less than equal to 40 per cent cooking salt. After addition of stabilizer, the cheese packed in hermetically closed containers was sterilized at at 80 to 95°C for 5 minutes, which prolonged the shelf life of the product to around 40 days.

Dehydration: For the US Army, a high quality dehydrated cottage cheese with 1.5 per cent moisture had been developed from frozen creamed and salted curds. The product is freeze dried to give a highly soluble powder suitable for reconstitution, and produces high quality fresh product upon the addition of clean water (Kosikowski, 1982). The frozen creamed curds after freeze drying under vacuum without thawing was stored under nitrogen in sealed tin cans with less than 2.0 per cent oxygen in the headspace gas. The shelf life of such dehydrated Cottage cheese was about 1 year at 22°C and 2 years at 5°C.
**Thermization:** Thermization destroys most of the psychrotrophic bacteria responsible for spoilage of Cottage cheese. Dajowiec et al. (1983) investigated that thermization of Cottage cheese at 65°C for 108 to 180 s extended its shelf life by 15 days at 4°C and concluded that the method was very suitable in extending the shelf life of the product without much deleterious effects on the organoleptic qualities alike other physical treatments.

**Microwave Treatment:** Tochman et al. (1985) investigated the thermal treatment of Cottage cheese in its package by microwave heating in order to the extend shelf life. Cottage cheese in polystyrene tubes or flexible pouches was heat treated at 37 to 82.2°C or 48.8°C, respectively using 0.5 or 2.8 kW microwave sources. The shelf life of the treated samples ranged from 7 to 42 days, optimum shelf life being obtained with packaged cheese heated to 48.8°C using 0.5 kW microwave power.

**Surface Pasteurization:** The most obtrusive deterioration of fresh soft cheeses, such as Cottage cheese, which results in unacceptable appearance, flavour and texture, is caused by growth of yeast and fungi such as *Penicillium*, *Geotrichum*, *Mucor* and *Alternaria*. To extend the shelf life of such cheeses, 'in-package' surface pasteurization of Cottage cheese was examined (Rosenthal et al., 1996). Results indicated that such treatment reduced levels of contaminating microorganisms by one order of magnitude to a depth of approximately 1 cm from the surface, and resulted in a shelf life of 3 to 4 weeks at 4°C.

**Hot packing and reduced washing:** Oamen and White (1984) investigated the effect of hot packing and reduced washing on the keeping quality of Cottage cheese. They observed that hot dressing at 71°C with a curd cream ratio of 52:48 without washing, gave a shelf life of 47 days at 5°C.

**Freeze-Drying:** Compact freeze-dried Cottage cheese, which is readily rehydratable to form an acceptable reconstituted Cottage cheese with good curd identity and long shelf life has been made by blending equal parts of a freeze-dried low milk fat Cottage cheese (dry curd containing <0.5 % milk fat before freeze-drying) and freeze-dried high milk fat Cottage cheese (creamed containing 4.0-4.5 % milk fat before freeze-drying) and compressing the resulting mixture at a pressure of 500 to 1000 lb/in² to a compression ratio of 3:1 to 4:1. This long life Cottage cheese with a shelf life of about 3 months was manufactured for the U.S. militia (Glickstein and Tuomy, 1978).

**Freezing and Brining**

Uncreamed Cottage cheese may be preserved for 90 days or longer by freezing (minus 1°C) or by brine storage (Kosikowski, 1982). However, freezing often resulted in graininess and curd shattering, particularly with rennet cheese. In brine salting curd remained intact. Brine-salted large curd cheese showed good keeping quality of at least six months without marked changes.
Gassing

CO₂ itself is antimicrobial agent acting as weak, organic acid (upon dissolution) penetrating plasma membrane and acidifying the cells interior. Other contributory factors thought to include changes in the physical properties of the plasma membrane adversely affecting solute transport; inhibition of key enzymes, particularly those involving carboxylation/decarboxylation reactions in which CO₂ is reactant, and reaction with protein amino groups causing changes in their properties and activities (Adams and Moss, 1995).

Kosikowski and Brown (1973) observed that CO₂ or N₂ flushing suppressed the growth of yeast, molds and psychrophils for upto 112 days. An excellent flavour and texture remained for upto 45 days.

Vacuum or gas packaging

Zimmerman and Kester (1960) reported that vacuum or gas packaging controlled Cottage surface spoilage by strict aerobic bacteria like pseudomonades, and yeast and molds. However, in another experiment, Sott and Smith (1971) reported that flavour scores and standard plate counts were not significantly higher than controls when 454-g polystyrene containers of Cottage cheese were gas-flushed in glass vacuum jars and stored for 11 to 12 days at 3°C.

Modified atmospheric packaging (MAP)

With the triumphal achievement of MAP in successful preservation of Mozzarella cheese, it has also been tried to increase the shelf life of Cottage cheese. Honer (1988) demonstrated that quality was maintained in cottage cheese packaged under MA for much longer period compared with air packaging. Effects of MAP on the growth of microorganisms in Cottage cheese were studied recently (Fedio et al., 1994). Cheese portions were packaged in high barrier mylar bags with different gases: 100 % N₂; 100 % CO₂; air; or 50 % CO₂ + 50 % N₂ and were stored at 5°C. Study indicated that MAP incorporating CO₂ could inhibit the growth of spoilage microorganisms in Cottage cheese packaged in high barrier containers. Results also showed that problems with *Listeria* might develop in cheeses packaged in air or N₂. It was recommended that Cottage cheese should be packaged in atmospheres containing high CO₂ level to attain shelf life of about 28 days. The direct-set Cottage cheese packaged in barrier containers was flushed with 100 % CO₂, 75 % CO₂, 25 % N₂, or air, and stored safely at 4°C for 28 days (Maniar et al., 1994).

Bio preservation

Preservation of Cottage cheese with microbial metabolites, an important category of biopreservatives, would be popularly acceptable both to the regulatory agencies as well as to the neurotic food faddists. Microbial metabolites can be produced *in situ* by adding the microorganisms along with Cottage cheese culture or in the Cottage
cheese dressing. Alternatively, biopreservatives can be added directly in Cottage cheese dressing.

**Microgard™**: Microgard™ is a skim milk that has been fermented by *Propionibacterium freudenreichii* subsp. *shermanii* and then pasteurized (Weber and Broich, 1986). The product has been approved by the FDA for food applications, such as Cottage cheese and fruit-flavoured yoghurt (Salih *et al.*, 1990) and is reportedly used as a preservative, e.g. in about 30 per cent of the Cottage cheese produced in the United States (Daeschel, 1989). Antimicrobial activity of Microgard™ has been well documented. It is active against Gram-negative bacteria including *Pseudomonas, Salmonella and Yersinia*, yeast and selected molds, but not against Gram-positive bacteria (Al-Zoreky *et al.*, 1991). The combination of inhibitor, organic acids, and diacetyl probably contributes to the overall preservation effect of Microgard™. The “natural” ways of extending shelf life of Cottage cheese include the addition of bifidobacteria to inhibit Staphylococci (Brivosa, 1987) or a pre-cultured skim milk product “Microgard™” (Salih *et al.*, 1990). The inhibitory effect of “Micrograd™” is attributed to a heat stable low molecular weight (about 7000 Daltons) peptide. “Microgard™” may be added to Cottage cheese at 0.4 per cent to prolong its shelf life (Weber *et al.*, 1986). It was also observed that addition of MicroGARD 400 at the level of 0.50% considerably improved the flavour as well as the aesthetic quality of the product during storage as well as extended the shelf life of cottage cheese from 12 to 26 days with an additional shelf life of 14 days, corresponding to an increase in keeping quality by ~117% in comparison to the control sample (Makhal and Kanawjia, 2003).

Mycostatin and Pimaricin: Nilson *et al.* (1975) reported that addition of antifungal agent mycostatin to the wash water or the dressing of Cottage cheese increased the shelf life of the product. With the addition of 0.0001 to 0.0005 per cent of the antifungal agents e.g. pimaricin or mycostatin to the cream dressing, they were able to extend the shelf life of Cottage cheese by two to 26 days at 4.5°C.

Tortorella *et al.* (1991) have succeeded in formulating the appropriate levels of antimicrobials, 0.02 per cent pimaricin or 0.02 per cent pimaricin in order to extend the shelf life of Cottage cheese from 21 days to over 35 days.

**Propionibacterial growth metabolites (PGM)**: The inhibitory effects of propionate (and acetate) produced by propionibacteria are potentiated by the low pH encountered of the environment where these strains are to be used. Moreover, propionic acid and its salts are also incorporated into bakery products to prevent mold growth and ropiness.

It is claimed that a metabolite material of propionibacteria, with a metabolite of MW >300, may be added to a food product to inhibit the growth of Gram-negative psychrotrophic bacteria, yeast, moulds, Gram-positive bacteria or *Listeria* (Ayres *et al.*, 1992). The metabolite material may contain <0.02 per cent propionic acid such
that there is insufficient propionic acid to inhibit microbial growth. It is claimed in a
US patent that a metabolite obtained from propionic bacteria can inhibit the growth
of Gram-negative psychrotrophic bacteria, *Listeria* spp., yeast and molds, when
added to food products, such as Cottage cheese (Mann, 1994). The incorporation of a
liquid PGM preparation into Cottage cheese caused significant reduction in spoilage
organisms and extension of shelf life. The addition of 1 per cent PGM to the dressing
used in Cottage cheese extended its shelf life by 6 to 9 days (Salih, 1986).

**Lactococcal growth metabolites (LGM):** Lactococci have been investigated to
produce bacteriocins also (Branen *et al.*, 1975 & Babel, 1977), such as nisin, lacticin,
diplococcin, lactococcin and others. Creamed Cottage cheese could be preserved
with that strain from which the naturally occurring 41 Mdal plasmid has been
removed, thus preventing the fermentation of lactose (Mann, 1991). Cottage cheese
manufacturers now often use live *Lc. lactis* subsp. *lactis* var *diacetylactis* cells in the
Cottage cheese dressing to maximize the shelf life of the cheese.

**Pediocin:** The ability of pediocin AcH, a bacteriocin produced by *Pediococcus
acidilactici* H to inhibit *Listeria* species in various foods, including Cottage cheese has
been investigated (Motlagh *et al.*, 1992). Sandine (1988) also reported that
*Pediococcus cerevisiae* metabolites inhibited *Listeria monocytogenes* and other
pathogens. Data suggest that these inhibitory activities may be due to bacteriocins,
though in some cases the activity is bacteriostatic rather than bactericidal.
Incubation of Cottage cheese products with *Pediococcus pentosaceus* NRRL-B-18229
has also been found to inhibit psychrotrophic spoilage bacteria (Matrozza *et al*.,
1988). Pediocin PA-1 has also been demonstrated to inhibit growth of *L.
monocytogenes* in Cottage cheese. It is suggested that bacteriocins be developed to
target a wider range of food spoilage to extend the shelf life and pathogenic
organisms (Marugg, 1991).

**Bifidobacteria**

Bifidobacteria strains (*Bifidobacterium infantis* NCFB 2255 and *B. breve* NCFB 2258)
are broad spectrum of antagonistic activity against both Gram-positive and
Gram-negative indicators, especially *Pseudomonas* spp. In experimental food trials
on the effects of *B. infantis* and *B. breve* upon growth of *Pseudomonas* spp. in Cottage
cheese, levels of *Pseudomonas* were reduced suggesting its exploitation in
preservation of Cottage cheese (O’-Riordan and Fitzgerald, 1998).

**Reuterin**

A broad-spectrum reuterin produced by *Lactobacillus reuteri* strain 12002 was
found to be inhibitory and bactericidal for *Listeria monocytogenes* and *Escherichia
coli* O157:H7 (El-Ziney and Debevere, 1998). Effects of reuterin on viability of these
pathogens in Cottage cheese have been investigated to find out a means of securing
safety and quality of the stored product. In Cottage cheese at pH 5.4, *L.
monocytogenes* increased by 0.4 log while *E. coli* O157:H7 decreased by 0.5 log in 21
days at 7°C; addition of reuterin (50-250 units/g) to the Cottage cheese reduced the viability of both organisms. As it has wide spectrum antimicrobial activity against both Gram-positive (Clostridium, Staphylococcus and Listeria) and Gram-negative (e.g. Salmonella and Shigella) microorganisms including yeast, fungi, and protozoa (De Vuyst and Vandamme, 1994a), it would provide better protection against pathogens as well as spoilage organisms.

**Nisin**

Nisin, produced by some strains of Lactococcus lactis spp. lactis is characterized by a strong bactericidal mode of action. Nisin disrupts semi permeable function of bacterial cell membrane and causes lyses of cells. It has now been exposed that the energy-transducing cytoplasmic membrane is the primary biological target site of nisin (De Vuyst and Vandamme, 1994c).

Benkerroum and Sandine (1988) reported on the use of nisin in Cottage cheese (2.55 mg/g) as an antilisteria agent. In another study, addition of 10 IU/g nisin effectively extended the shelf life from 21 days to over 35 days without any signs of visible spoilage (Tortorello et al., 1991). Nisin when added along with EDTA acts strong antimicrobial agents against Gram-negative microorganisms including psychrotrophic bacteria, yeast and molds, mostly responsible for the spoilage of Cottage cheese.

**LP-system**

Some UK scientists have made an interesting study on the preservative action of an activated LP system in chilled stored Cottage cheese. It has been concluded that LP system could provide an effective natural preservation system to increase safety and improve stability of Cottage cheese (Mann, 1991). Naturally activated LP-system affords protection against spoilage of Cottage cheese by inhibiting the growth of psychrotrophic bacteria.

**Fruit mix**

Addition of Fruit mix to creamed Cottage cheese curd at the rate of 25 per cent to the top, rather than the bottom of the container has been observed to inhibit psychrotrophic bacteria, yeast and molds by limiting available oxygen in the Cottage cheese, thereby increasing shelf life as well as enhancing acceptability (Campbell, 1989). In the investigation, pineapple, peach or strawberry fruit flavours were added at 25 per cent by weight to the bottom of 6-oz Styrofoam cups, which were then filled with creamed Cottage cheese curd (Campbell, 1989). On the other hand, filling was added to the top of containers of commercially processed creamed Cottage cheese curd. Not only did fruit enhance the flavour of Cottage cheese, it masked off-flavours produced by microbial metabolism near the end of product shelf life. Shelf life of fruit-flavoured samples was extended by about 7 days (fruit at
the bottom of the container) or 14 days (fruit at the top).

**Manufacture of cottage cheese**

Recently at NDRI a technology has been developed for manufacture of Cottage cheese employing dual acidification technique following the procedure (Makhal and Kanawjia, 2005) as outlined below:

![Diagram of Manufacture of Cottage Cheese]

**Fig. 2. Manufacture of Cottage Cheese**
Addition of κ-carrageenan

High heat treatment while using κ-carrageenan

25-50% HCl soln → Cold acidification

Slow rate (0.7°C/min) of tempering (15°C)

GDL → Hydrolysis of GDL

Slow rate (0.7°C/min) of tempering (20°C)

TSPP addition → Renneting

Curd setting (25°C)

Cutting (1/3 inch cheese knives)

Cooking (35°C/30 min)

Washing (Thrice: 25, 15 and 8°C)

Draining (45min)

Dressing

(22% ripened curd dressing ≅ 4% fat in final product)

Packaging (Polystyrene Cup)

Storage (4-5°C)

Fig. 1 Flow diagram for manufacturing direct acidified cottage cheese employing dual acidification technique

References


• 385.


Fermented milk products are a class of traditional milk products produced since vedic times in India. 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). Many of the traditional fermented products are produced at homes by adopting boiling, fermentation process and in certain cases with culinary intervention. Some of the fermented products are naturally acidic, some are sweet as in the case of Mishti Dahi and Srikhand. Some are salty like Majjige, Raita and some are garnished with spices as in the case of Raita and Kadhi. All these products are prepared on regular basis in many of the households throughout the country. The preparation also varies from season to season and many of these products are served in cold especially during summer seasons.

The milk production in India is estimated at 110 million tonnes, valued at approximately 1200 billion Indian rupees. Almost about 7% of this milk is converted into various fermented products valued at around 15,000 crores. The share of organized sector in this category is less than 10 per cent and hence there is immense scope for the upgradation of manufacture of these products by the dairy and food industry. Some of the specialized products having commercial value with scope for process upgradation are detailed in the present document.

**Curd rice:** Curd rice is one of the popular items in the diet of people of Southern India. It is consumed almost every day with every meal throughout the year. It is estimated that almost 16-30% of the milk purchased by the households in the region is converted to Dahi which is then used to prepare curd rice or consumed with rice after mixing. It is also served along with the meal by industrial catering systems. Hence, curd rice forms one of the widely used milk and cereal based products of the region, having vast scope for production and sale on commercial scale.

The nutritional value of food depends not only on the nutrient content but also on the digestibility and assimilability. The nutritional characteristics of curd or dahi, a lactic fermented product with high nutritive value have been reviewed extensively (Vaghela and Kilara, 1992). Thus curd rice forms not only a widely consumed staple food item but also forms one of the nutritive items of every day diet, in the Southern States of India.
**General characteristics**

Curd rice is a food containing cooked rice, Dahi (fermented milk product), approximate quantity of salt and sometimes a variety of spices incorporated into the product. The addition of spices not only adds to the flavour and taste but also improves its shelf life to a certain extent. It has a mild acidic flavour and taste and is considered to be nutritionally superior. The nutritional superiority could be ascribed to the nutritive value of fermented milk.

Eventhough the curd rice is being consumed as an item of diet since a long time, there is no documented scientific information on the product. Hence, a study was initiated at the Institute and a part of the information relating to the quality of the product available in the market is presented.

![Flow diagram of curd rice manufacture](Fig.1)

**Fig.1 - Flow diagram of curd rice manufacture**
**Market quality:** The quality of the curd rice available in the market based on a market survey conducted in the city of Bangalore indicated following characteristics (Rashmi et al 2003)

Curd rice available in the market is generally salted and taste may vary from mildly sour to extremely sour and generally visible whey separation is observed in the product. During summer months, many a times off flavours are noticed in the product. Almost all samples were found to be garnished and other ingredients like coriander leaves, curry leaves, chillies both green and dry red, seasoning materials, cucumber pieces, pomegranate seeds, ginger etc., were found in the samples. The shelf life rarely exceeds 24 hours when stored under refrigerated conditions. Based on extensive study the process of production was optimized and the flow diagram of manufacture is furnished (Fig.1). The composition of curd rice and the material balance are presented in Tables 1 & 2.

**Table: 1 Composition**

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
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</tr>
<tr>
<td>Protein</td>
<td>1.90</td>
</tr>
<tr>
<td>Fat</td>
<td>2.19</td>
</tr>
<tr>
<td>Ash</td>
<td>1.03</td>
</tr>
<tr>
<td>Carbohydrates (by difference)</td>
<td>9.92</td>
</tr>
<tr>
<td>Total Solids</td>
<td>12.04</td>
</tr>
<tr>
<td>Water Activity</td>
<td>0.994</td>
</tr>
<tr>
<td>Acidity</td>
<td>0.48-0.54</td>
</tr>
</tbody>
</table>

**Table: 2 Ingredients required for 100 kgs**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>9.30</td>
</tr>
<tr>
<td>Water</td>
<td>49.00</td>
</tr>
<tr>
<td>Milk</td>
<td>40.00</td>
</tr>
<tr>
<td>Salt</td>
<td>1.00</td>
</tr>
<tr>
<td>Green chillies</td>
<td>0.30</td>
</tr>
<tr>
<td>Coriander Leaves</td>
<td>0.30</td>
</tr>
<tr>
<td>Ginger</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**Kalan:** Kalan is another popular product of Southern India prepared predominantly in the state of Kerala. The product is one of the classic examples of a traditional product prepared by hurdle technology. The details of the products and the optimized process of flow diagram of manufacture are presented below (Surendranath et al 2009).
Kalan – A regional dahi based product

From the information collected on dahi based traditional foods for the region, it was observed that *kalan*, a popular traditional product from Kerala, has a reasonable shelf life.

In the traditional method preparation of *kalan*, dahi is concentrated by boiling it along with turmeric and curry leaves. Cooked vegetable (elephant yam and unripened banana colloquially known as *nendra bale*), coconut paste and spices are added to the concentrated dahi and boiled further. The consistency of *kalan* could vary from free flowing to the semi solid with suspended vegetable pieces. The flavour of the product is contributed by fenugreek, turmeric and curry leaves. The product on storage at room temperature becomes unacceptable after 3 days due to flavour deterioration and mould growth.

A procedure was standardized to optimize the process for the preparation of the product with enhanced shelf-life.

Milk (Fat 3%, SNF 9%) was boiled and converted to dahi (1% LA). Dahi (2 l) was then concentrated (1:2.5) along with curry leaves (25 g) and turmeric (1.5 g) and was blended to obtain a smooth texture. Pieces of yam (400 g) and peeled raw banana (200 g) were shallow fried in ghee with pepper powder, turmeric powder and salt. The fried vegetable pieces were pressure cooked and a paste made of coconut gratings (150 g), green chillies (30 g), cumin seeds (5 g) was added and boiled for 5 min followed by addition of fenugreek powder (7 g). The product was then seasoned with mustard, red chillies and curry leaves in coconut oil and boiled for 2 min. The product on sensory evaluation was observed to be highly acceptable.

*Kalan* could be stored at room temperature for 3-5 days and about 20 days in refrigerator. The major problem affecting the shelf-life was found to be mould growth. In attempts to enhance the shelf-life, the product (200 g) was packed in flexible multilayer pouches. The pouches were kept in boiling water bath for 10 min. This treatment gave a shelf-life of more than 3 months to the product when stored at room temperature.

During storage, no change in the moisture was noticed. An increase in the acidiy of the product was observed during storage. However, this did not affect the sensory quality of the product.

Microbial behavior with respect to total plate count (TPC), coliform, yeast and mould, and staphylococcal counts in *kalan* stored at room temperature was studied at 15 days interval up to 90 days. Total plate count of the product at 0 day ranged from as low as $4.7 \times 10^2$ to $1.35 \times 10^3$ cfu/g of the different batches and reached to a maximum $8.8 \times 10^4$ cfu/g on 30th day of storage. Beyond 30 days of storage, the product showed declining trend in TPC which was $6.3 \times 10^2$ cfu/g on 90th day of storage. Analysis of coliform counts revealed non-detection of the organisms at any
stage of storage indicating that GMP was followed during the manufacture of the product. Investigations on yeast and mold counts indicated a marginal increasing trend with extended period of storage, to a maximum, upto 60 cfu/g. *Staphylococci* and *S. aureus* were not detectable even at 15th day of storage indicating the unfavourable environment of the product for growth of spores and enterotoxigenic staphylococi.

**Cucumber cultured buttermilk drink:** Cultured buttermilk drink, produced from *dahi* by appropriate dilution, is a refreshing beverage and popular in southern states. Health benefits of buttermilk can be enhanced by the use of certain additives like spices, herbs, vegetables and fruits. Cucumber (*Cucumis sativus*), a commonly used vegetable in green salads and culinary products, is rich in phytochemicals and dietary fibre and is known to possess cooling and diuretic properties. An attempt was made to blend fresh cucumber juice with *dahi* to produce a cucumber – buttermilk drink. Cucumber (1 kg) washed and blanched with hot water (at 85°C for 5 min), cooled to room temperature was macerated in a mixer grinder. The pulp was diluted with one portion of water to obtain cucumber juice (2° Brix). Boiled and cooled toned milk was fermented with a mixed starter culture (*Lb* + *St*) to obtain dahi with an acidity of 1.4 % lactic acid. Dahi thus obtained was blended with cucumber juice and water (1:3). To enhance the taste and aroma salt @ 0.7 % and steam distillate of ginger @ 6 % were added to the drink. The product with a pleasant light green colour was accepted well during sensory evaluation. The cucumber – cultured buttermilk drink had 3.5 % total solids, 0.7 % fat, 1.0 % protein, 1.0 % lactose and 1.2 % ash. The product was evaluated for its yeast and mold count and was found to be free from the same. The product packed in glass bottles kept well for 15 days under refrigerated storage.
**Kadhi:** Kadhi is one of the ethnic fermented products, which is popular in most parts of our country and is produced by using various ingredients like dahi, Bengal gram flour, spices etc. The limited shelf life of kadhi at room temperature is an impediment for taking the product for wider marketing. Attempts were made to produce long life kadhi at room temperature by adopting retort processing. Technology for production of kadhi was standardized with respect to various parameters like fat & snf, acidity of the curd, lactic culture and Bengal gram flour level. Two hundred gms of kadhi was filled in flexible retortable pouches and were subjected to F0 treatments of 3.0 and 5.0 in a over pressure rotary retort sterilizer. The samples were stored at 37°C for 5 weeks and subjected for chemical, sensory, rheological and microbiological analysis at 7 days interval. No significant sensory changes were observed between the two samples treated at F0 values of 3.0 and 5.0. The kadhi samples were found to be acceptable at the end of 5 weeks without any significant sensory, chemical, rheological and microbiological changes. Based on the results obtained, it is concluded that a commercially sterile kadhi in Ready-To-Use (RTU) form can be prepared by packing it in a flexible retortable pouch and subjecting it to F0 treatment of 3.0 in a over pressure rotary sterilizer system. The optimized process flow diagram for manufacture is detailed in Fig. 2 and the chemical composition in Table-3.

**Fig.2 Flow Diagram for the production of Kadhi (Manohar, 2005)**

---

<table>
<thead>
<tr>
<th>Receiving Milk</th>
<th>↓</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standardization</td>
<td>↓</td>
</tr>
<tr>
<td>Heat Treatment</td>
<td>↓</td>
</tr>
<tr>
<td>Cooling</td>
<td>↓</td>
</tr>
<tr>
<td>Inoculation</td>
<td>↓</td>
</tr>
<tr>
<td>Incubation</td>
<td>↓</td>
</tr>
<tr>
<td>Dahi</td>
<td>↓</td>
</tr>
<tr>
<td>Stirring</td>
<td>↓</td>
</tr>
<tr>
<td>Bengal gram flour &amp; Chilli powder</td>
<td>↓</td>
</tr>
<tr>
<td>Seiving</td>
<td>↓</td>
</tr>
<tr>
<td>Paste preparation with water</td>
<td>↓</td>
</tr>
<tr>
<td>Mixing</td>
<td>↓</td>
</tr>
<tr>
<td>Heating with continuous stirring</td>
<td>↓</td>
</tr>
<tr>
<td>Addition of turmeric powder &amp; salt</td>
<td>↓</td>
</tr>
<tr>
<td>Boiling (10 min.)</td>
<td>↓</td>
</tr>
<tr>
<td>Seasoning with Ghee, Onion, Spices etc.</td>
<td>↓</td>
</tr>
<tr>
<td>Kadhi</td>
<td>↓</td>
</tr>
</tbody>
</table>

---
### Table-3: Chemical Composition of Kadhi

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Range %</th>
<th>Mean*± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>80.57-80.62</td>
<td>80.595±0.025</td>
</tr>
<tr>
<td>Total Solids</td>
<td>19.38-19.43</td>
<td>19.41±0.055</td>
</tr>
<tr>
<td>Fat</td>
<td>4.92-4.96</td>
<td>4.94±0.044</td>
</tr>
<tr>
<td>Protein</td>
<td>4.33-4.37</td>
<td>4.35±0.045</td>
</tr>
<tr>
<td>Ash</td>
<td>1.81-1.83</td>
<td>1.82±0.014</td>
</tr>
<tr>
<td>Carbohydrates (by difference)</td>
<td>8.27-8.32</td>
<td>8.29±0.040</td>
</tr>
</tbody>
</table>

### Conclusion

There are several traditional fermented products in Southern parts of India. Some major products other than “Dahi” are detailed in the current presentation. Some of the products are prepared and consumed regularly and also some of the products are commercially prepared. There is scope and opportunity for the commercialization of the other traditional fermented products.

### References

Refrigeration plays an important role in preservation of perishable commodities, i.e. to safeguard these against spoilage, particularly the food items including milk and other dairy products. The milk and dairy products although produced continually must be preserved against the time of distribution and kept until required by the consumers. This time may be weeks or even months after processing.

All these physical and chemical changes and bacterial growth causing spoilage of dairy products can be considerably retarded in their activities by their exposure to low temperature conditions and proper level of humidity and purity of surrounding air. These favourable conditions are generated in cold storage to retard spoilage and preserve the stored products for longer period. Thus the cold storages are designed according to favourable conditions of temperature, humidity and purity of air required for the type of dairy product to be stored. The basic principle of producing cold or low temperature in cold storage is same by using a vapour compression refrigeration system.

**Principles of cold storage**

Cold storage is the method of preserving perishable commodities including dairy products in their fresh and wholesome state for extended periods by providing and controlling proper temperature and humidity conditions within the storage compartments, because normal atmospheric conditions of temperature and humidity are seldom at a level conducive to the safe and prolonged storage of perishable foods. It is necessary that artificial methods be provided to produce such an environment. This safe environment is formed of various favourable conditions of air inside the cold storage given below:

**Types of conditions inside a cold storage**

**Temperature:** The first main condition of air required in a cold storage is 'low temperature'. The low temperature retards or arrests most of the spoiling activities like bacterial growth in dairy products.

**Humidity:** It is the moisture content of air in cold room. It is also an important parameter and required to be kept in a certain range. Very low humidity means the product will lose its moisture due to increased evaporation in dry air. Very high humidity means the growth of moulds/ bacteria will take place. However, effect of humidity is lowered down either by packaging the products or by wrapping these with moisture resistant paper.
**Motion of air:** It means that the cold air should not be stationary but should flow continuously over the products for better heat transfer. It is ensured by suitably designing the cooling coil and blower.

**Purity of air:** It is also an important parameter because impure air may badly affect the quality of food products stored in. Suitable measures are taken to ensure purity of inside air. In this way the major consideration is made for temperature, humidity, motion and purity of air in a cold storage. But in what range the temperature and humidity should be, is decided and designed based on some prior considerations as mentioned below:

**Prior considerations in deciding inside conditions of a cold storage**

In actual, cold storage conditions are determined first by the type of food to be stored and second by the length of time such foods are to be stored.

**Type of food:** In general, types of foods are divided into two groups:

- Foods in which living process continues, i.e. these absorb oxygen and produce respiration heat. Fruits and vegetables come in this category. The low temperature exposure has only a retarding effect on these foods.
- Non-living foods such as meat, fish and dairy products that are highly susceptible to the activity of spoilage agents. These products deteriorate rapidly unless the drastic preventive measures of preserving these are taken.

**Storage time:** Second consideration is the length of time for which dairy products are required to be stored/ preserved. It decides chiefly that the products are to be stored in frozen or unfrozen state. The storage period is less for unfrozen products and longer for frozen products. However, this rule is not so simple because the storage period, even in frozen state, varies considerably from product to product. Freezing temperature also vary for different dairy products.

Table 1 shows the approximate temperatures and Relative humidity used for curing various other types of cheese, which are common in America. With the exception of the soft ripened cheeses such as Camembert and Liederkranz, freezing of cheese results in undesirable texture changes. This can be very serious as in the case of cream cheese where a mealy, pebbly texture results. Other types such as brick or Limburger undergo a slight roughening of texture which is undesirable but which still might be acceptable to certain consumers.

Table 2 shows the freezing points of common varieties of cheese as determined by Watson and Leighton, Bureau of Dairy Industry, USDA. The freezing points reported here are substantially lower than the temperatures at which undesirable texture changes are known to take place in commercial practice. As a general rule cheese should never be subjected to temperature under 29 F. The oiling off point of all types of cheese except process cheese is about 68 F.
When cheese is held above its oiling-off point the fat leaks from the body and rapidly develops rancidity. Processing cheese protects the cheese from oiling off. By heating the bulk cheese to temperatures of 140 to 180 °F, and through the incorporation of emulsifying salts, a more stable emulsion is formed than in the natural or non-processed cheese. Process cheese will not oil off even at melting temperatures, because of the temperatures used in processing. Process cheese is essentially a pasteurized product. Microorganisms causing changes in the body and flavour of the cheese during cure are largely destroyed hence there will be practically no further flavour development consequently; the maximum permissible temperature for the storage of process cheese is considerably higher than the other types.

**Table 2: Freezing points of various cheeses**

<table>
<thead>
<tr>
<th>Cheese</th>
<th>Freezing point, (°F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brick</td>
<td>16.3</td>
</tr>
<tr>
<td>Cheddar</td>
<td>8.8</td>
</tr>
<tr>
<td>Cottage</td>
<td>29.8</td>
</tr>
<tr>
<td>Limburger</td>
<td>18.7</td>
</tr>
<tr>
<td>Process (American)</td>
<td>16.6</td>
</tr>
<tr>
<td>Process (Swiss)</td>
<td>17.5</td>
</tr>
<tr>
<td>Roquefort</td>
<td>3.7</td>
</tr>
<tr>
<td>Swiss, domestic</td>
<td>14.0</td>
</tr>
<tr>
<td>Swiss, imported</td>
<td>14.7</td>
</tr>
</tbody>
</table>

**Refrigeration of cheese drying rooms**

Cheddars, daisies and similar round styles that are to be dried prior to waxing, enter the cooler at approximately room temperature and sufficient refrigerating capacity must be provided to reduce them to drying room temperature. This product load may be taken as one ton for each 6000-8,000 kg per day. Product load in a cheese drying room is usually small compared to total room load and extreme accuracy in figuring product load is not warranted. To determine the peak-refrigerating load in a cheese drying room, the factor to be remembered is that the peak cheese production may coincide with periods of high ambient temperature. Also, these rooms normally open directly into the cheese make room where both temperature and humidity are quite high and traffic in and out of the drying room may be heavy. Therefore, ample allowance for door losses should be made. Two to
three air changes per hour are quite possible during the flush season. Humidity control during the winter presents certain problems. Since most of the refrigeration load (during the peak season) is due to insulation losses and warm air entering through the door, the refrigerating units may not operate enough during cold weather to take up the moisture given by the cheese, resulting in excess humidity and improper drying of the cheese. Within certain limits this can be overcome by reducing the speed of the unit fans and lowering the backpressure. If there are several units in the room, the refrigeration may be turned off on some. (Fans should be left running to insure uniform conditions throughout the room.) If these adjustments are not sufficient, or if automatic control of humidity is desired, it will be necessary to use reheat coils in the air stream leaving the units. These may be electric heaters, steam or hot water coils or hot gas from the refrigerating system. A heating capacity of 15-20% of the refrigerating capacity of the units is usually sufficient. A humidistat may be used to operate the heater when the humidity rises above the desired level. The heater should be wired in series with a second room thermostat set to shut it off if the room temperature becomes excessive. Because of variation in size and shape of drying rooms it is impossible to generalize on air velocities and capacities. Airflow should be regulated so that cheese feels moist for the first 24 hr, after which it becomes progressively dryer and firmer.

**Components of a cold storage**

**a). Cold room:** It is an insulated room, cabin or compartment with an insulated door for loading and unloading the products to be stored. Size of the cold room varies considerably depending on the variety and amount of products to be stored. There may be more than one cold room in a dairy plant operating at different temperatures. Generally separate cold rooms are designed for different dairy products of varying temperature requirements.

**b). Cooling coil/diffuser:** Cooling coil also called as evaporator/evaporator coil, is the only component of refrigeration machine which is fixed inside the cold room, as the name suggests, it cools the products kept inside the cold room. Depending on the size of cold room/store, one or more cooling coil (evaporator coil) with air diffusing system is fixed inside the cold store. In air diffusing system a fan or blower pulls the air over the cooling coil and diffuses this cold air in whole space as shown in fig.1. It is fixed at heat height on the suitable wall of cold store. However, height may vary as per the requirement. Maximum distance is maintained between the cooling coil and cold store door. Sometimes air ducts hanging from roof may also be used for proper distribution of cold air in the whole space. The cooling coils may be of two types as 'direct expansion evaporator coils’ or 'chilled water coils'.

1. **Direct expansion cooling coil:** In this type of cooling coil, the refrigerant is directly expanded and evaporated. That's why it is also called as evaporator. During evaporation, the refrigerant absorbs heat of the surrounding air and produce
cooling effect. It is more efficient and cheap method of refrigeration. But the drawback is that any leakage of refrigerant like ammonia can spoil the food products stored.

2. **Chilled water coil:** In this system chilled water from Ice bank is circulated through the cooling coil. It is preferred where the purity of air is highly important i.e. where there is a risk of spoilage of all the foodstuffs because of the presence of traces of refrigerant in cold air due to any leakage.

c). **Compressor room:** In a large sized cold storage of a dairy plant, a separate room for compressors is provided. This room is situated as near to cold room as possible to minimize the piping and insulation cost and also to enhance the overall performance of the plant. Generally, there is more than one reciprocating type compressor. Separate compressors are there for cold rooms working at different temperatures because of difference in suction pressure. However, the discharge pressure and temperature of all the compressors is same. So the discharge line of all the compressors is merged into a single line and connected to condenser.

![Fig 1(a) Blower Type Cooling oil](image)

![Fig 1(b) Blower Type Cooling Coil](image)
d). Condenser and receiver: As the discharge temperature of the all the compressors is same, a single condensing unit is employed. Either a water- cooled condenser with cooling tower or an evaporative condenser is used. The condenser is situated in an open space nearest to condenser room/cold room to minimize the piping cost. The receiver is also situated near to condenser to collect the compressed and condensed refrigerant gas and then to supply it to cooling coil through control/ expansion valves.

e). Expansion/control valves: Separate expansion valves and control valves are fixed for different cold rooms. These are fixed on the outside of that wall of cold room on which the cooling coil is fixed. In this way these are connected to cooling coil across the wall. With dry expansion evaporator, thermostatic expansion valve is used and with flooded evaporator, float control valve is used.

f). Refrigeration piping and refrigerant: All the major components of refrigeration system are connected to each other through pipes. Steel pipes are used with ammonia as refrigerant and copper pipes are used with Freon refrigerants. The pipe connecting the cooling coil with compressor, also called as suction pipe, carries the low temperature refrigerant gas. So insulation is provided on it. All other pipes are bare. As generally the compressor, condenser and receiver are situated at some distance away from the cold room, the length of connecting pipes carrying refrigerant is also very large. Because of this, the quantity of refrigerant is large. So, the cost of refrigerant is important, as costly refrigerant cannot be used in large quantity. Generally ammonia gas is most accepted refrigerant in the cold storage of a dairy plant. Ammonia is cheap, easily available and also very good refrigerant in performance. The typical layout of cold storage of a dairy plant is as shown in figure.

g). Safety devices: In addition to the major components, many safety devices are also installed in a cold storage plant. Some of the safety devices and their function are as discussed below:

1. Low pressure cut out
2. High pressure cut out
3. Temperature Indicator/Controller
Fig 2: Typical layout of a cold storage
Low pressure cut-out: In a refrigeration plant, when sometime the cooling load decreases or evaporator becomes ineffective due to some other reason, the evaporation of refrigerant liquid in the evaporator coil decreases. Due to decrease in evaporation of refrigerant, the pressure of refrigerant vapours also decrease in the suction side of compressor. This low pressure also called high vacuum may cause damage to the equipment, i.e., any pipe or joint can burst. It also puts heavy load on compressor. This high vacuum is avoided by the use of low-pressure cutout, which switch of the compressor automatically as soon as the pressure in evaporator decreases below a certain value. As shown in fig.3, it consists of an electric switch, which is operated by the combined force of a spring and pressure force in the suction line. At normal suction pressure, it remains in OFF position. But when suction pressure decreases below a certain limit, it comes in ON position and activates a relay, which switches off the compressor. It is a mandatory fitting with compressor of a refrigeration plant.

High pressure cut-out: In a refrigeration plant, the condenser effectiveness may decrease sometimes due to failure of cooling water supply to condenser or by deposit of dust or sludge etc. on its surface or by the presence of air with refrigerant vapour in the condenser. When condenser effectiveness decreases, the vapours are less able to condense and due to more vapours continuously coming from compressor, their pressure may increase than the nominal value. This high pressure is dangerous to the equipment i.e., compressor and condenser. Hence to avoid high pressure, a high-pressure cut out is connected to discharge line, which switch off the compressor automatically in case the pressure in condenser exceeds to an upper limit. As shown in fig.4, it consists of an electric switch, which is operated by the combined force of a spring and pressure/force in the discharge line. At normal
discharge pressure, it remains in OFF position. But when discharge pressure increases above a certain limit, it comes in ON position and activates a relay, which switches off the compressor. It is a mandatory fitting with compressor of a refrigeration plant.

**Fig. 4 High Pressure Control**

**Temperature controller:** In a cold storage, once the food/dairy products stored come at a low storage temperature, there is no more heat to be extracted from the foodstuffs kept in cold storage. However, some heat may come inside from the hot surrounding but its rate is very less due to effective insulation all around the cold room. It is also very less as compared to heat extraction rate of the refrigeration plant. Thus, there is no further need to keep on running the refrigeration plant until the inside temperature again increases above a certain limit. So, for that purpose some automatic method is required which switch on and switch off the compressor depending on the temperature inside the cold room. An electronic temperature controller is generally used for this purpose. When the refrigeration plant is operated, it senses the temperature of cold room continuously through a probe hanged inside. As soon as the required low temperature is reached inside the cold room, it automatically activates a relay switch, which switches off the compressor. This temperature, at which the compressor is switched off, is called cutout temperature. Now again, when inside temperature starts increasing due to inflow of some heat from surrounding and also through operating the door of cold room, and when it crosses an upper limit, temperature controller starts the compressor. This upper limit temperature is called cut-in temperature. In this way, temperature controller is the most important control device in a refrigeration plant.
Conclusions

All the physical and chemical changes and bacterial growth retard considerably if we keep the milk and dairy products at low temperature and proper level of humidity in surrounding air. On storing at these favourable conditions of temperature and humidity, products can be preserved for a long time. The preservation time depends on the type of dairy products and temperature at which it is stored. Dairy products can be stored either in frozen state or unfrozen state depending on required preservation time. Once these conditions of temperature and humidity are decided, cold storages are accordingly designed. Mainly the calculations are made for heat requires to be extracted from the products. Some more heat, which enters in the cold storage through various unavoidable sources, is also added. Total heat required to be extracted and sometimes moisture also decides the cooling load of refrigeration unit.
GENETIC ENGINEERING OF DAIRY STARTERS AND THEIR APPLICATIONS IN DAIRY PRODUCTS

Rameshwar Singh, Surajit Mandal and R. P. Singh

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. They cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. Also, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exo-polysaccharides, and several enzymes is of importance. In this way they enhance shelf life and microbial safety, improve texture, and contribute to the pleasant sensory profile of the end product. Lactic acid bacteria, propionibacteria, surface-ripening bacteria, yeasts, and moulds are used as starter cultures for manufacturing of various fermented milk products. Starter cultures have a multifunctional role in dairy fermentations. 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 flavour 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 flavour 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 exo-polysaccharides 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 flavour production, lack of associated off flavours, bacteriophage tolerance, ability to produce flavour during cheese ripening, salt tolerance, exo-polysaccharide production, bacteriocin production, temperature sensitivity, 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). This functionality can contribute to microbial safety, or offer one or more organoleptic, technological, or nutritional and health advantages to the food. Promising examples are lactic acid bacteria 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. The genetic alterations of LAB may also lead to strains with improved traits. These may be either attractive for the manufacturer of fermented foods, or have benefits for the consumer. LAB in which the genetic material has been altered by recombinant DNA technology in a way that does occurs naturally for instance by point mutations or small deletions are considered as genetically modified LAB. Due to the considerable economical importance of LAB, many groups are now actively working on these bacteria using an array of genetic tools. Many chromosomal genes of interest have been characterized. *Lactococcus lactis* IL1403 was the first LAB to have been completely sequenced. LAB can also be engineered to function as cell factories. Cell metabolism can be engineered to massively produce metabolites of interest such as food additives and aroma compounds. They were also shown to be able to produce proteins with applications to health or the development of new vaccines. It might be expected that the development of knowledge of the interaction between certain LAB and the human host will allow to better exploitation of the expected natural potential to improve health. Genetic alterations result in a change of the genetic code of the micro-organism that may affect the transcription and translation processes and, consequently, may influence metabolic processes in the cell.

**Metabolites produced by lactic acid bacteria**

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 *Lactobacillus, Lactococcus, Leuconostoc* and *Sterptococcus*. LAB fermentation yields primarily lactic acid, which plays a vital function in safeguarding food products. LAB metabolism beneficially affects 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. In addition, many lactic acid bacteria produce compounds of human nutritional value as regular end products in their metabolisms, including some B-vitamins. Many strains of LAB and bifidobacteria produce other metabolites that promote
human health. Bioactive peptides generated from milk proteins as a result of their proteinase and peptidases activity and production of CLA from linoleic acid by strains of lactobacilli and bifidobacteria. For instance, the meat isolates *L. sakei* CTC 494 and *L. curvatus* LTH 1174 turned out to be promising novel bacteriocin producing starters for sausage fermentation, whereas *L. amylovorus* DCE 471 seemed more suitable as a starter for industrial sourdough fermentation. *E. faecium* RZS C5 has potential to be used as a bacteriocin producing co-culture for food fermentations. Moreover, modelling indicated how exopolysaccharide production by *S. thermophilus* LY03 in yoghurt may be optimised by adjusting the process conditions.

### Table 1: Starter cultures and their applications

<table>
<thead>
<tr>
<th>Starter bacteria</th>
<th>Functionality</th>
<th>Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactococcus lactis</em> ssp. lactis, <em>L. lactis</em> ssp. cremoris, <em>Enterococcus spp.</em>, <em>L. carvatus</em>, <em>L. sakei</em>, <em>P. acidilactici</em>, <em>E. faecium</em>, <em>L. plantarum</em>, <em>L. ruteri</em>, <em>Streptococcus thermophilus</em></td>
<td>Bacteriocin production</td>
<td>For bio-preservation of foods</td>
</tr>
<tr>
<td>Several EPS producing lactic acid bacteria (lactobacilli streptococci, and lactococci)</td>
<td>Exo-polysaccharides production</td>
<td>Good body and texture of low fat fermented dairy products, Prebiotics for probiotics, Special health benefits</td>
</tr>
<tr>
<td>Galactose fermenting lactobacilli and streptococci</td>
<td>Galactose utilization</td>
<td>Low level of galactose in fermented milk products, Low browning in mozzarella cheese</td>
</tr>
<tr>
<td>Lactose negative <em>L. delbrueckii</em> ssp. <em>bulgaricus</em></td>
<td>Prevention of over acidification in yoghurt</td>
<td>Good body and texture, Prevention of curd syneresis</td>
</tr>
<tr>
<td>Autolysing lactic acid bacteria</td>
<td>Enhanced proteolytic and lipolytic activities</td>
<td>Accelerated ripening of cheeses, Production of bioactive milk peptides</td>
</tr>
<tr>
<td>Mannitol, sorbitol producing lactic acid bacteria (Leuconostoc spp.)</td>
<td>Production of low calorie sugars in fermented milks</td>
<td>Reduced calorie misthi dahi &amp; lassi, Special health benefits</td>
</tr>
<tr>
<td>Probiotic lactic acid bacteria</td>
<td>Different functional attributes</td>
<td>Probiotic and functional dairy dairy products and other foods</td>
</tr>
<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, Natural way of vitamin enrichment of fermented milks and dairy products</td>
</tr>
</tbody>
</table>

**Genetics of lactic acid bacteria**

Because of its singular economic importance as the starter bacterium for industrial production of various fermented dairy products, and the relative ease by which it can be handled in the laboratory, much of our current understanding of genetics in dairy lactic acid bacteria have come from study of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*. Four types of genetic elements that have been characterized at the
nucleotide sequence level in *L. lactis* and to a lesser extent in other dairy LAB. These are plasmid DNA, transposable elements, bacteriophages and, most impressively, the bacterial chromosome.

**Plasmid DNA**

Plasmids are extra chromosomal, autonomously replicating DNA molecules that exist independently of the bacterial chromosome. Molecular and genetic studies of bacterial plasmids have yielded extraordinary insight into cellular mechanisms for DNA replication, gene transfer, gene expression, and genetic recombination. Plasmids have also played an integral role in development and evolution of recombinant DNA technologies for many organisms, including dairy LAB. The number of copies at which a particular plasmid species exists within a bacterium (i.e., its copy number) varies widely and can range from as few as one or two to tens or even hundreds of molecules. Under most conditions, plasmid-coded functions are not essential to host survival (exceptions involve properties such as antibiotic resistance that confer a selective advantage under specific environmental conditions), but they may allow the cell to compete better with other microorganisms that share their ecological niche. Therefore, if a daughter cell loses a particular plasmid species through plasmid replication or segregation errors, it will usually continue to grow and may even predominate over its wild-type population. Loss of the plasmid will, however, result in permanent loss of any trait encoded by that plasmid.

The first reports of plasmid DNA in LAB were published in the early 1970s by researchers working with *E. faecalis* and *S. mutans*. Among food-grade LAB, it was the long-standing observation that many *L. lactis* dairy starters permanently lost their acid or flavour producing phenotypes (and the fact that the frequency of these events increased under plasmid curing conditions) that served to stimulate the first inquiries into the plasmid biology of these organisms. Lactococci are an especially fertile source of plasmid DNA, and that gene for many of this bacterium’s industrially important traits are encoded by plasmids. The latter discovery enlivened worldwide interest in LAB plasmid biology and genetics, and we now know that plasmid DNA is a frequent component of the genome in lactococci, leuconostocs, oenococci, pediococci, and some lactobacilli. Plasmids have also been identified less frequently in other food-grade LAB, including *Carnobacterium* spp., *S. thermophilus*, *Tetragenococcus* spp. and *Weissella* spp. The rich diversity of plasmid species in LAB is fortuitous, because it provides a ready source of extra-chromosomal replicons to support development of gene-cloning vectors.

**Transposable elements**

Transposable elements are discrete sequences that have the ability to move from one site to another in DNA. Three types of mobile genetic elements have been found in LAB - insertion sequences (IS), transposons and introns. By virtue of their
mobility, these elements promote genetic rearrangements that can affect the organization, expression, and regulation of existing genes. In addition to insertional inactivation of target or adjacent genes, transpositional elements can also induce expression of flanking genes. Transposons and IS elements also promote more extensive forms of intragenomic rearrangements such as co-integrations, inversions, and deletions.

Comparative genomic analysis of *L. lactis* has revealed that an inversion encompassing approximately half of the chromosome in strain ML3 is the result of homologous recombination between two copies of IS905. Transposable elements can contribute to genetic variation in bacteria by facilitating horizontal gene transfer between different strains, species, and genera. IS elements were involved in horizontal transfer of genes for exo-polysaccharide production between *L. lactis* and *S. thermophilus*. From a more practical perspective, transposable elements can be useful tools for molecular analysis of LAB genetics, physiology, and metabolism, and for development of integrative gene cloning vectors.

### Table 2: Transposable elements in lactic acid bacteria

<table>
<thead>
<tr>
<th>Host and element name</th>
<th>Size (bp)</th>
<th>Inverted repeat (bp)</th>
<th>IS Family</th>
<th>Copies per genome</th>
<th>Host range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus</em> ISL1</td>
<td>1256</td>
<td>40</td>
<td>IS3</td>
<td>1-3</td>
<td><em>L. casei subsp. casei, L. zeae</em></td>
</tr>
<tr>
<td>ISL2</td>
<td>858</td>
<td>16</td>
<td>IS5</td>
<td>4-21</td>
<td><em>L. helveticus</em></td>
</tr>
<tr>
<td><em>Lactococcus lactis</em></td>
<td>808</td>
<td>18</td>
<td>IS6</td>
<td>1-20</td>
<td><em>L. plantarum, L. lactis</em></td>
</tr>
<tr>
<td>ISS1</td>
<td>808</td>
<td>18</td>
<td>IS6</td>
<td>1-20</td>
<td><em>L. plantarum, L. lactis</em></td>
</tr>
<tr>
<td><em>Leuconostoc</em> IS1070</td>
<td>1027</td>
<td>28</td>
<td>IS30</td>
<td>&gt;15</td>
<td><em>Leuconostocs lactis</em></td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>1411</td>
<td>24</td>
<td>ISL3</td>
<td>ND</td>
<td><em>S. thermophilus</em></td>
</tr>
<tr>
<td><em>thermophilus</em> IS1193</td>
<td>1411</td>
<td>24</td>
<td>ISL3</td>
<td>ND</td>
<td><em>S. thermophilus</em></td>
</tr>
</tbody>
</table>

### Chromosome

Genes encoding all of the essential housekeeping, catabolic, and biosynthetic activities of the cell are housed in the chromosome. The map of genes have confirmed that individual species and even strains may differ in genomic size and organization, and show that all LAB characterized to date possess a single and circular chromosome.

### Bacteriophages

Bacteriophages, or phages for short, are viruses that attack and destroy bacterial cells. The inhibitory effect of these obligate parasites on dairy starter bacteria has been recognized and their destructive impact on the cheese and fermented dairy products industries has focused worldwide attention on molecular genetics and evolution of LAB phages. Because industrial fermentations with *L. Lactis* and *S. thermophilus* starters suffer greatest economic losses, current understanding of LAB
phage biology stems largely from phages infecting these two species. However, several groups have described bacteriophages infecting other industrially important LAB species, including many dairy lactobacilli, and some of these phages have even been characterized at the genome sequence level.

**Gene transfer mechanisms**

Modern genetics flows from the ability to manipulate living cells in ways that heritably alter the physiological properties. This achievement has become possible through discovery and refinement of gene transfer mechanisms in bacteria and higher cells. Four types of gene transfer processes that have been established in dairy LAB: transformation, transduction, conjugation. Although each has played some role in the genetic analysis of dairy LAB, transformation and, to a lesser extent, conjugation has clearly emerged as the most useful methods for genetic manipulation in these species.

**Transformation**

Transformation is the process wherein free DNA molecules are introduced into cells. Many bacteria, including some species of non-dairy streptococci, can assume a “competent” state that allows them to take up DNA from their environment. This ability is determined by a set of unique genes that encode proteins for extracellular DNA binding, uptake, and integration. Expression of host competence genes is induced when the concentration of a host-secreted, competence-stimulating peptide (i.e. competence pheromone) in the medium reaches a critical threshold. Natural competence has reported that the *L. lactis* genome appears to contain a complete set of competence genes. In the absence of natural competence, the most effective method for transformation in most bacteria is electroporation. When cellular membranes are exposed to a high-voltage electric field, they become polarized and a voltage potential develops across the membrane. Electroporation technology is based upon the discovery that when this potential exceeds a certain threshold, localized breakdown of the membrane forms pores that render the cell permeable to extraneous molecules. The first reports of transformation by electroporation (electro-transformation) in dairy LAB had been successfully applied to *L. lactis, S. thermophilus*, and many species of *Lactobacillus* and *Leuconostoc*.

**Transduction**

Transduction is a form of gene transfer which can result from inadvertent packaging of host DNA within a bacteriophage virion during phage replication. Genetic exchange is affected when the phage particle injects this DNA into another bacterium. Phage-mediated gene exchange in LAB was first described using of tryptophan biosynthesis and streptomycin resistance markers by a virulent *L. lactis* bacteriophage. Plasmid transduction by virulent or temperate phages has also been demonstrated in *S. thermophilus, L. salivarius*, and *L. gasseri*, but even though this form of gene transfer helped to establish important genetics principles in *L. lactis*, it
has not found similar applications in other food-grade LAB. Much of the current disinterest in transduction as a tool for genetic studies or improvements in LAB stems from the relatively narrow host range of transducing phages and, more importantly, development of more effective gene transfer systems such as conjugation and transformation.

**Conjugation**

Conjugation is a natural form of gene transfer in bacteria that requires physical contact between viable donor and recipient cells. Genes required for conjugative transfer are typically located on self-transmissible plasmids and conjugative transposons, but transfer of non-conjugative plasmids can also be affected via processes termed donation and conduction. The former process applies to non-conjugative plasmids that possess a specific sequence, called the origin of transfer (oriT), that is required for DNA mobilization. Transfer of these plasmids rely only upon trans-acting gene products from a conjugative element and not on co-integrate formation between the non-conjugative and conjugative elements. In contrast, plasmid transfer by conduction does require co-integration, because the non-conjugative molecule lacks a functional oriT. As a genetics tool for dairy LAB, conjugation has proved especially useful to study plasmid biology in *L. lactis*. An important outcome of this work has been the finding that many industrially important traits, including lactose and casein utilization, bacteriophage resistance, and bacteriocin production, can be transferred by conjugation. Conjugation to genetically enhance bacteriophage resistance in commercial *L. lactis* starter cultures has been applied.

**Genetic modification**

- Cloning systems
- Chromosome modification systems
- Expression systems

**Cloning systems**

"Food grade" and are allowed to be introduced into our food. In general, food grade systems have to contain only genetic elements that are as safe as the host. It is more or less accepted that these elements must have originated from bacteria that already have a long history of use in food. In most cases, these genetic elements derive from plasmids and genes from bacteria of the same species, to provide a “self-cloning system”. They should be well characterized and not contain antibiotic resistance markers, and not require the use of harmful compounds. In addition to these safety issues and legal constraints, food grade systems have to meet minimum requirements for stability under industrial conditions and scale of use and have to be applicable in an efficient and cost-effective manner.
A number of plasmid vector systems have been developed using the origin of replication of natural plasmids combined with food-grade selection markers, such as the wide hostrange pWV01 or pVS40 plasmids the narrow host range pCI305 in lactococci, or pFR18 in *Leuconostoc mesenteroides*. Integrative plasmid strategies have also been developed using phage or transposon integrative systems, such as those of the A2 phage of *L. casei* [16], mv4 of *L. delbruekii* and TP901-1 in *L. lactis*. Systems based on homologous recombination by single cross-over of non-replicative plasmids were used as integrative vector by removing part of their replication functions.

These strategies necessitate the use of selective markers that will allow their selection and maintenance in the host. Two kinds of markers can be defined, those that are selectable because they confer a new phenotype, and those that restore an impaired function. In the first class are sugar utilization markers such as sucrose or xylose genes, bacteriocin resistance genes such as those conferring insensitivity to nisin or lactacin F or metal such as cadmium. For the second type of marker, any function necessary for cell viability and that can be conditionally inactivated to produce auxotrophic mutants can be used. A system based on the inactivation of lacF, a lactose gene, has been developed in *L. lactis*. The present list is not exhaustive, but shows that a number of food-grade vectors and markers are now available in different LAB.

**Chromosomal modification systems**

Although many vectors are now available, the systems described in the previous section have several disadvantages - i) copy number of plasmids may vary, ii) plasmids may be lost in the absence of marker selection, iii) plasmids may be structurally instable etc. These vectors require the introduction DNA in addition to that of the desired gene. Tools are also available to insert genetic constructions by allelic replacement in the chromosome. This method has several advantages over replicative or single cross-over integration vectors. In particular, allelic replacement allows stable DNA insertion or genetic modifications without leaving any foreign DNA other than that desired. Allelic replacement occurs by double cross-over between two regions of homology flanking the modification and the corresponding regions on the chromosome. A thermo-sensitive plasmid-based system has been developed allowing gene replacement by a two-step procedure. A mutation in the gene encoding plasmid replication protein of the natural plasmid pWV01 has been selected, allowing maintenance of the plasmid at 30°C, but not at 37°C. This plasmid can direct homologous integration in the *L. lactis* chromosome when it carries a chromosomal DNA fragment of sufficient length (about 500 bp or more). Lastly, this plasmid can also be used to select food-grade mutants containing a single IS element as new DNA fragment in the genome.
Expression systems

In addition to cloning systems, gene expression systems have been developed allowing the controlled expression of homologous or heterologous genes. Most of these systems were developed in *L. lactis*, such as those based on promoters controlled by sugar (lactose operon promoter), by salt (gadC promoter), by temperature upshift (tec phage promoter), pH decrease (P170) and phage infection (phi31-promoter). A dose-dependent system of induction is also available using sub-lethal concentrations of nisin in *L. lactis* and this system was extended to some other LAB. Sugar-dependant expression systems have also been developed in other LAB such as lactobacilli. Although very interesting for the production of heterologous proteins, inducible systems are not always easy to manage under industrial conditions, especially if a constant level of production is required for metabolic control, for example. In this case, a well-defined constitutive promoter with the desired level of expression may be more efficient. A system of synthetic promoters allowing the constitutive and defined level of expression of downstream genes been created recently and could, in principle, be applied to any bacterial species.

Genetically modified lactic acid bacteria and their applications

Lactic acid bacteria are naturally diverse and strains belonging to the same species may have very different properties. These differences are largely exploited, offering a wide range of strains that can then be combined as a function of the required processes and products. However, one may want to combine a specific trait of one strain with another strain that has a background well adapted to a particular process. This could be of particular importance if only a single strain is available for a given process, precluding the use of alternative strains in case of phage attack. There is thus a need to be able to combine strain characters in order to produce reliably fermented food products of high quality. In some cases, the desired modification could be restricted to the mutation of a single gene, a process that can occur spontaneously, sometimes at relatively high frequencies. Thus, lactococcal variants in lactose metabolism, citrate uptake and proteolytic activity can easily be obtained by simple screening procedures, because the genes necessary for these metabolic pathways are encoded on segregationally unstable plasmids.

The number of traits that could be modified by such an easy method is quite limited, and more efficient screening strategies should be set up to select mutants arising at frequencies of 10^-6 or lower, which is approximately the level of spontaneous mutation of a gene in the chromosome. In some cases, screening can be facilitated by colour reactions, such as the use of X-gal to select strains devoid of beta-galactosidase activity. This screening strategy was used to select *L. bulgaricus* strains unable to efficiently ferment lactose and do not acidify yogurt after fermentation has finished. Indeed, upon storage, yogurt pH may drop below a value of 4.0, increasing the acid and bitter taste of the product, and thus degrading its
initial organoleptic quality. This post acidification process is mainly due to \textit{L. bulgaricus} lactose metabolism. Lactose deficient strains are still able to grow in association with \textit{S. thermophilus}, the second yogurt starter strain. Use of such a strain allows the production of yogurt that can be kept for months at 4 °C without a significant drop of the pH. Use of mutagenic compounds such as EMS or \textit{N}-methyl-\textit{N'}-nitro-\textit{N}-nitrosoguanidine may be used to increase the rate of recovery of mutations. LDH mutants having a mixed pattern of fermentation, and producing increased amounts of acetoin and diacetyl, were selected by such mutagenesis strategy. However, additional mutations may occur necessitating careful testing for other important traits. To circumvent this problem, genetic engineering can be used advantageously. Improving the flavor and the flavororal stability in buttermilk through metabolic engineering of \textit{L. lactis} subsp. \textit{diacetylactis} is possible.

<table>
<thead>
<tr>
<th>Lactic acid bacteria</th>
<th>Gene level</th>
<th>Beneficial effect</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{L. bulgaricus}</td>
<td>lacZ</td>
<td>Reduction of lactose utilization</td>
<td>IS mediated deletion</td>
</tr>
<tr>
<td>\textit{L. lactis}</td>
<td>ldh and others</td>
<td>Increased diacetyl production</td>
<td>Induced random mutagenesis</td>
</tr>
<tr>
<td>\textit{L. lactis}</td>
<td>aldB</td>
<td>Increased diacetyl production</td>
<td>Spontaneous random mutagenesis</td>
</tr>
<tr>
<td>\textit{L. lactis}</td>
<td>ribC</td>
<td>Increased riboflavin production</td>
<td>Induced random mutagenesis</td>
</tr>
<tr>
<td>\textit{S. thermophilus}</td>
<td>gal operon</td>
<td>Galactose fermentation</td>
<td>Spontaneous random mutagenesis</td>
</tr>
</tbody>
</table>

Diacetyl is responsible for the butter flavor in many fresh dairy products such as butter, cream and buttermilk. However, even if its presence at low concentration is sufficient to confer this typical aroma, diacetyl is highly labile and its loss results in a flat taste of the products. The main pathway for diacetyl production is a two step synthesis from pyruvate. The first step is common to valine biosynthesis and acetoin catabolism pathways through the reaction of two pyruvate molecules to give alpha-acetolactate catalyzed by alpha-acetolactate synthetase. Diacetyl is then formed by a chemical oxidation occurring spontaneously at low rate. However, in \textit{L. lactis}, alpha-acetolactate is also actively decarboxylated by alpha-acetolactate decarboxylase into acetoin, a compound that does not confer the desired flavor. Inactivating \textit{aldB}, the gene encoding alpha-acetolactate decarboxylase, should thus increase the availability of alpha-acetolactate for chemical oxidation.
Gene technology allows the production of similar aldB mutants by direct allelic replacement using an appropriate thermo-sensitive vector. This approach was also adopted to obtain food-grade mutants of *L. lactis* resistant to phages by the inactivation of the phage infection protein (*pip*) involved in phage adsorption and DNA injection. Similar approaches could also be used in other LAB species such as *S. thermophilus* where the inactivation of the phosphoglomutase gene enhances polysaccharide production and that of urease genes reduces delay in the acidification in milks containing high amount of urea.

Table 4: Genetic modifications of lactic acid bacteria by recombination of genes

<table>
<thead>
<tr>
<th>Lactic acid bacteria</th>
<th>Source of gene</th>
<th>Gene level</th>
<th>Beneficial effect</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. lactis</em></td>
<td><em>L. lactis</em></td>
<td><em>aldB</em></td>
<td>Increased diacetyl production</td>
<td>Homologous recombination</td>
</tr>
<tr>
<td><em>L. lactis</em></td>
<td><em>L. lactis</em></td>
<td>Lacticin encoding genes</td>
<td>Lacticin production</td>
<td>Conjugation</td>
</tr>
<tr>
<td><em>L. lactis</em></td>
<td><em>L. lactis</em></td>
<td>Prt+ and Lac+ <em>L. lactis</em></td>
<td>Increased proteolysis and acid production</td>
<td>Conjugation</td>
</tr>
</tbody>
</table>

**Constructing modified strains with genes from other LAB**

The modified bacteria could be considered as mutants equivalent to bacteria that might already exist naturally. However, introducing new DNA encoding new information into the bacterial cell could lead to wider strain diversity. We will examine examples of engineered strains obtained by the introduction of new genes from other bacteria of the same or a different species of LAB. To obtain strains with increased proteolytic properties, the genes encoding PepN, PepC, PepX and PepI peptidases of a highly proteolytic *L. helveticus* strain or PepI, PepL, PepW, and PepG
from *L. delbrueckii* were transferred into *L. lactis* using a food-grade cloning system. It is expected that such recombinant bacteria producing an additional peptidolytic enzyme activity may make an important contribution to proteolysis during maturation of cheese, for example, by shortening the ripening period and allowing the production of special cheeses (e.g. reduced-fat cheeses) with improved characteristics.

Another example of gene transfer between LAB is provided by the construction of bacteriophage resistant strains. LAB that are used repetitively and massively in industrial productions can be highly subject to infections with bacteriophages. This leads to the lysis of the starter and thereby the arrest of fermentation. The products obtained then do not have the desired quality, and may eventually be lost. The origin of bacteriophages is still discussed, since they may come from raw products such as milk that had contact with environmental farm bacteria including wild LAB, from inoculum (mostly if it is not properly produced), from the factory itself, or from the evolution of remnant phages present in the starters. Selection of bacteriophage resistant strains is thus an ongoing task of starter producers. Research on bacteriophage resistance determinants in certain strains led to the characterization of a number of resistance mechanisms. Phage resistance systems may interfere with phage adsorption, phage DNA injection, phage replication, transcription, RNA translation, protein assembly and phage packaging. These mechanisms are often carried out by mobile elements such as plasmids and transposons suggesting that lateral transfer of these genes occurs under pressure of phage infection. Some high level resistance plasmids were shown to carry more than one resistance mechanisms. To improve phage resistance, one could rationally combine these mechanisms as a function of their target in phage development and of the phages present in the factories.

In addition to these natural resistance mechanisms, recent advances in the knowledge of phage biology has allowed the generation of new weapons by targeting specific steps in phage development. For example, to lower phage proliferation, it has been proposed to introduce a further phage replication origin that competes with that of the phage. Another strategy is to induce the expression of a lethal gene upon phage infection or to massively produce antisense mRNA against essential phage genes. The most important drawback of these systems is their narrow range of action. Lastly, DNA shuffling, exploiting the properties of a type I restriction enzymes could also generate new restriction/modification mechanisms. Phage and cellular genes involved in cell lysis were proposed to be used to construct cells that will lyse at an appropriate moment during cheese making to improve cheese ripening. Indeed, whereas starter lysis is a major problem during fermentation, late cell lysis allows the release of many enzymes into the cheese matrix, improving in particular the degradation of peptides. This degradation allows cheese to be made less bitter (due to the reduction of some bitter peptide), and
contain more free amino acids (precursors of aroma). Several approaches have been proposed to provoke controlled lysis of starter bacteria, including the use of autolytic strains, bacteriocin producing starters and phages. Several of these approaches are not easy to optimize, or may even be seen as unacceptable by industry because of the use of phages that may contaminate other processes in the factory. Engineered strains producing phage derived lyasin and holin proteins or bacteriocin under the control of an inducible promoter have been constructed. Increased lysis of the cells was obtained and cheese trials have shown that under some conditions this lysis may allow an increased aroma production. However, additional work is needed to optimize the strains for industrial use. In addition to accelerating cell lysis for cheese ripening, heterologous bacteriocins could also be produced to eliminate undesirable contaminant bacteria. An *L. lactis* strain containing the complete *eps* cluster from *S. thermophilus* Sfi6 is able to produce an exopolysaccharide of similar size to that of the native strain. However, its composition differs suggesting that an additional chromosomal copy was required for its complete synthesis. Similar experiments carried out with the *eps* cluster from *S. thermophilus* Sfi39 allowed the production of an EPS similar to the one produced by Sfi39. These examples show that complete complex biosynthesis pathways can be introduced in LAB, and in the above mentioned case, would have application to feed production or in the improvement of the texture of fermented food.

**Table 5: Genetic modifications of lactic acid bacteria by cloning of genes**

<table>
<thead>
<tr>
<th>Lactic acid bacteria</th>
<th>Source of gene</th>
<th>Gene level</th>
<th>Beneficial effects</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. lactis</em></td>
<td><em>L. helveticus</em></td>
<td>pepN, pepX, pepC</td>
<td>Improved proteolytic systems for cheese ripening</td>
<td>Food grade vector cloning</td>
</tr>
<tr>
<td><em>L. lactis</em></td>
<td>Lytic phage phi31</td>
<td>Anti sense phage RNA</td>
<td>Inhibition of phage replication</td>
<td>Vector cloning</td>
</tr>
<tr>
<td><em>L. lactis</em></td>
<td><em>S. thermophilus</em></td>
<td>abiA, abiG</td>
<td>Phage abortion</td>
<td>Vector cloning</td>
</tr>
<tr>
<td><em>L. lactis</em></td>
<td><em>L. plantarum</em></td>
<td>MtlD</td>
<td>Increased mannitol production</td>
<td>NICE</td>
</tr>
<tr>
<td><em>L. lactis</em></td>
<td><em>L. lactis</em></td>
<td>nisRK, nisFEG</td>
<td>Nisin Z production</td>
<td>Vector cloning</td>
</tr>
<tr>
<td><em>L. lactis</em></td>
<td><em>S. thermophilus</em></td>
<td>EPS-gene cluster</td>
<td>EPS production</td>
<td>Vector cloning</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td><em>B. subtilis</em></td>
<td>phyC</td>
<td>Phytase activity</td>
<td>Vector cloning</td>
</tr>
</tbody>
</table>

**Compensation for metabolic defects**

The potential of LAB to correct metabolic defects has been relatively less studied. However, it has been known for a long time that fermentation by LAB reduces lactose intolerance. Although it is controversial, it has been suggested that bacterial lactase could naturally supplement the human enzyme in cases of deficiency. The idea that LAB could compensate for enzyme deficiency is thus not new. Recently, lipase from *S. hyicus* was produced massively intracellular in *L. lactis* in order to
deliver high quantities of this enzyme in the jejunum. Indeed, *L. lactis* was shown to be able, under certain conditions, to pass through the stomach and massively lyse in the jejunum where the lipase should be delivered. Oral treatment with *L. lactis* expressing this lipase was carried out in a pig model where pancreatic insufficiency was induced by ligation of the pancreatic duct. The coefficient of fat absorption was significantly higher after consumption of lipase-expressing *L. lactis* than after consumption of the control strain showing that this strategy could in principle be applied to compensate for enzyme deficiency.

**Other applications**

In addition to food and health applications, genetically modified lactic acid bacteria has been used as improved biosensors for the detection of biocides in milk. Initially, a commercial product made use of a *S. thermophilus* strain particularly sensitive to antibiotics to detect possible contamination of milk that might perturb fermentation. This test requires a few hours to assess whether the metabolic activity of the strain is inhibited. To shorten it, luciferase genes were introduced in this strain and their expression optimized. Finally, using reduced light production in highly bioluminescent *S. thermophilus*, test times could be significantly shortened compared to the previous commercial test utilizing the related non-bioluminescent strain. This GMO is an improved test kit to detect antibiotic residues in milk.

**Conclusion**

Selecting for strains with interesting properties to be used as new, functional starter cultures may lead to an improved fermentation process and an enhanced quality of the end product. Rational selection of appropriate strains is crucial. Because of legislation and marketing reasons, the industrial application of carefully selected natural food isolates with functional properties seems attractive. Of course, selection of starter cultures must not only aim at expressing functional properties, but also at eliminating undesirable side effects. One of the challenges of using wild-type strains will be to allow large-scale production of fermented foods without losing their unique flavour and other traits. In the new generation of fermented foods, LAB with diverse physiological and metabolic traits is combined. Their metabolic and technological properties are often different from those of traditional starter cultures, so that appropriate production processes must be developed. Novel insights into the metabolism of LAB offer perspectives for the application of a new generation novel starter cultures. The process of introducing novel traits into LAB by the addition, substitution, removal, or rearrangement of defined DNA sequences, including the DNA sequences used for the maintenance or transfer of the new DNA into the recipient strain could lead to the generation of LAB with novel genetic properties and a modified cellular behaviour. However, unintended effects, that could be a consequence of the modification, could also occur in the newly generated LAB. Predictable effects are a foreseeable and direct consequence of the intended
genetic alterations and unexpected effects are caused by unintended genetic alterations, or they may occur as a consequence of predictable effects. LAB have an unblemished history of safety in food fermentation, but, as was discovered later, may produce unfavourable amines under some conditions. Evidently these strains should not be used in preparation of foods without a profound safety assessment that, especially in this case, also investigates the actual concentrations of the harmful compound to which the consumer will be exposed. Broad evaluation of the nature of novel foods derived from GM-LAB, which should be considered as a specific group of GMOs, could be a starting point to bridge the gap between industry, consumers and green groups. This may lead to acceptance of GM-LAB derived novel foods to provide for a better quality of life in today's society.

References

To make cheese what one would minimum need is milk, starter culture and rennet (a milk coagulant). This is a very general and layman’s conception when cheese making was Craft and not Science as it is today. Today apart from starter bacteria, internal and external moulds and the milk coagulants, there are a large no. of additives including salt (i.e. sodium chloride) are used either in cheese milks, cheese curds or cheeses to achieve set goals related to cheese quality.

**Additives for use in cheese under PFA 2005**

The PFA rules 2005 do not permit addition of (a) Stabilizers either singly or in combination expressed as anhydrous substances, (b) Thickeners and Modifying Agents singly or in combination, (c) Modified Starches singly or in combination and (d) Flavours in Cheese/Sliced/Cut shredded cheese, Processed Cheese or Processed Cheese Spreads.

It permits addition of colours (natural - singly or in combination) like Curcumin (100 mg/kg max), Riboflavin (100 mg/kg max), Chlorophyll (100 mg/kg max), Carotene, Natural Extract (100 mg/kg max), Annatto extracts on Bixin/norbixin basis (50:50 ratio) (10-50 mg/kg max, Normal to orange coloured) in Cheese/Sliced/Cut shredded cheese, Processed Cheese or Processed Cheese Spreads. Whereas Beta carotene (100 mg/kg max), Beta apo carotenal (35 mg/kg max) and Methyl ester of beta apo 8 carotenoic acid (35 mg/kg max) are permitted only in Cheese/Sliced/Cut shredded cheeses.

Calcium carbonates and Magnesium carbonates in sufficient quantity are permitted in Cheese/Sliced/Cut shredded cheeses as acidity regulators.

With respect to preservatives, Sorbic acid, Sodium sorbate, Potassium sorbate, Calcium sorbate (expressed as Sorbic acid) and Nisin are permitted respectively at 3000 mg/kg max and 12.5 mg/kg max in Cheese/Sliced/Cut shredded cheese, Processed Cheese or Processed Cheese Spreads. Addition of Propionic acid/Sodium Propionate/Calcium propionate, expressed as Propionic acid singly or in combination at 3000 mg/kg max is permitted in Cheese/Sliced/Cut shredded cheese only.

Sorbic acid, Potassium sorbate and Calcium sorbate expressed as Sorbic acid singly or in combination at 1 g/kg max and Pimaricin (Natamycin) at 2 mg/dm. sq. surface, not present in depth of 5 mm are permitted for surface/rind treatment in Cheese/Sliced/Cut shredded cheese only.
In Cheese/Sliced/Cut shredded cheese anticaking agents like (a) Cellulose, (b) Carbonates of Calcium & magnesium (c) Phosphates of Calcium & Magnesium (d) Silicates of calcium, magnesium, aluminium or sodium or Silicon dioxide and (e) myristates, palmitates or stearates of aluminium, ammonium, calcium, potassium or sodium are also permitted at 10 g/kg max level.

Acidifying agents like (a) Citric acid (b) Phosphoric acid (c) Acetic acid (d) Lactic acid and (e) Sodium bicarbonate/Calcium carbonate expressed as anhydrous substance singly or in combination are permitted at 40 gm/kg maximum level with emulsifiers in Processed Cheese or Processed Cheese Spreads.

PFA rules also permit addition of emulsifiers viz. (a) Potassium salt of mono/di and poly phosphoric acid, (b) Calcium salt of mono/di and poly phosphoric acid, Sodium salt of mono/di and poly phosphoric acid (c) Sodium citrate (d) Potassium citrate and Calcium citrate and (a) Citric acid with sodium hydrogen carbonate & Calcium carbonate or (b) Phosphoric acid with sodium hydrogen carbonate and or calcium carbonate either singly or in combination at levels not exceeding 40 g/kg except that added phosphorus compound should not exceed 9 g/kg calculated as phosphorus in Processed Cheese or Processed Cheese Spreads.

However, FAO/WHO Committee defines no quantitative restrictions on the use of Sodium chloride, Phosphoric acid, Riboflavin, Lactic acid, Propionic acid, Pimaricin (antimycotic), Citric acid, H₂O₂ and catalase and Chlorophyll including Copper chlorophyll. However that does not mean any quantity can be added.

The additives having limits of usage under FAO/WHO include (a) Annatto and β-carotene alone or in combination, <0.06 % (w/w), (b) Benzoic acid and the sorbic acid salts (Na, K or Ca) are limited to coagulant preparations, i.e. ‘rennet’, (c) Calcium chloride, <0.02 % (w/w), (d) Enzymes of animal or vegetable origin for flavour development, <0.1 % (w/w), (e) Nisin, <0.25 % (w/w), (f) Phosphate, <0.002 % (w/w), (g) Sodium Hydrogen Carbonate, Calcium Carbonate <3 % (w/w) (used in Quarg or sour milk or lactic curds), (h) Sodium or calcium nitrate, <0.02 % (w/w), (i) Sorbic acid or its sodium or calcium salts, <0.3 % (w/w) calculated as sorbic acid (antimycotic uses) and (j) Whey proteins, when added to cheese, <20 % in fat free dry matter of the cheese.

The additives normally used in cheese processing include (1) Salts to restore the calcium balance in milk (2) Acids that are used alone or as supplement to lactic acid, (3) Colours and bleaching agents, (4) Salts inhibitory to undesirable organisms, (5) Emulsifying salts (6) Flavours in spices and herbs, (7) Smokes (8) Beverage flavours - beer, wine liqueurs, etc and (9) Other goods, i.e. vegetables, soy solids, other forms of milk solids, other edible foods, fish, ham, etc.
Salts to restore calcium balance of milk

Calcium salts

The calcium balance between the soluble, colloidal and complexed is very delicate and successful coagulation depends on this balance. Thus when (1) cheese milk inherently has disturbed balance or lacks calcium content, (2) lack of balance or disturbance caused due to either severe heating of milk during pasteurization or chilling and cold storage at 4-5°C for long periods causes dissociation of β-casein, (3) Certain microbial and vegetable milk coagulants are used or (4) dilution of milk with water has been done addition of calcium salt to the milk is generally practiced.

Calcium may be added to milk in forms such as (i) calcium chloride, (ii) dibasic calcium phosphate – recommended for use with pepsin rennet, (iii) lime water and (iv) calcium lactate.

The beneficial effects of addition of CaCl₂ on RCT and gel strength is believed to be due to (i) increase in Ca⁺⁺, (ii) increase in CCP and (iii) decrease in pH.

Calcium chloride as a standardized solution is the most common way of adding the salt to milk to allow easy distribution. Accurate quantities must be used. Usually, not more than 0.02 % of calcium chloride is needed for satisfactory coagulation. Addition of too much CaCl₂ leads to (1) disassociation of αs-casein - κ-casein complex so that the αs-casein no longer has the protection from the κ-casein and a precipitate forms and (2) a hard, unyielding curd, bitter in flavour and harsh body due to retention of too much CaCl₂.

Phosphate salts

Phosphate in the form of sodium phosphate has been used to restore the salt balance (calcium / phosphate) where very disturbed conditions have prevailed. Milk from cows fed on potato silage does not coagulated by rennet until phosphate is added to the milk. From 60 to 80 % of the calcium in milk and from 50 to 60 % of the phosphate is retained in hard cheese. In soft cheeses more of the salts are lost and therefore, less calcium and phosphate are retained.

Acidulants

Lactic acid

Lactic acid is the most widely used acidulant in cheese making. It can be produced in situ by lactic acid bacteria by natural flora of milk. This method lacks consistency of performance and many a times results in off flavours and body and texture defects in curd and/or cheese. It can also be produced by pure/defined cultures. The use of such cultures gives consistently good quality curd and thus cheese.
**Other acids**

Acids of food grade quality such as lactic acid, glacial acetic acid, lime juice/citric acid; D-glucono-delta-lactone, phosphoric acid, etc. are used to bring about coagulation of milk in manufacture of certain cheese varieties.

Glacial acetic acid is used in Queso Blanco cheese – a Latin American variety (1.25 litres diluted to 10 litres and used for 450 litres of milk). Vinegar (e.g. 0.03 %) is also used for Mozzarella cheese manufacture. Lime juice/citric acid is used In India in manufacture of Bandal cheese and Chhanna. D-glucono-delta-lactone causes progressive acidification of milk due to its hydrolysis, which leads to the formation of a smooth, homogeneous coagulum. However, cheeses produced with D-glucono-delta-lactone lack characteristic cheese flavour due to absence off enzymes contributed by starter bacteria hence, not much used in commercial practice for manufacture of ripened varieties. It is used in aseptic cheese making and in manufacture of certain soft un-ripened cheeses.

**Cheese colours**

The colour of milk and of cheese is an important factor in the consumer appeal of the product. It is a common practice to add extra colour to pale (light) coloured milks to give cheese an attractive and appetizing appearance, to supplement the intensity of the natural colour where it is perceived to be weak and to ensure batch-to-batch uniformity. The two colours of milk of importance are:

**Riboflavin:** Pigment contained in serum portion (whey) with a green yellow fluorescence. It gives greenish tinge to curds. However, during cheese making most of it is lost in whey. Therefore, as a colouring agent its contribution to colour of cheese is negligible.

**Carotenoids:** Impart deep yellow orange colour to milk and thus to cheese and hence are significant. There is large variations in initial intensity of this colour in milk depending on a number of factors e.g. species, breed, feed, season, etc. Thus to maintain uniformity of colour through out the year, cheese makers restore to use of appropriate colouring matters, viz. annatto, β-carotene and saffron [used in cheese like Box (Germany), Caciotta (Italy) and Luneberg (Austria)].

**Annatto cheese colour**

Annatto cheese colour is by far the most widely used colour. It is a colour of vegetable origin. It is extracted using alkali (NaOH/KOH) from the fresh seeds of a South American shrub, *Bixa orellana*. The total amount of pigments in the seeds is reported to be 0.76 g/100 g. The pigment in annatto is the acid bixin, which in the alkaline extract becomes norbixin. The colour is composed of tints of yellow and red units, and in cheese becomes a protein dye attached to the casein.
Bixin (Mol. wt. 394.49) is a mono methyl dicarboxylic acid and is soluble but in vegetable oils (suitable for colouring butter or very high fat cheese). Salts of norbixin, which are not soluble in oil but soluble in water, are produced from bixin, by saponification with ammonia and NaOH/KOH, for use in cheese.

Annatto is very susceptible to oxidation. The agents such as H₂O₂, air, SH groups in ripening cheese and copper and iron act as catalysts in oxidation of annatto pigment. Thus, bleaching of the red colour in patches in cheese is frequently found in poor quality, moist or contaminated curd.

The amount of annatto colour to be added depends on number of factors such as (i) variety of cheese (The hue, yellow or orange, depends particularly pH of the cheese to being made, (ii) type and strength of colour used, (iii) initial intensity of colour present in milk and (iv) colour intensity desired in the resultant cheese - dictated by the variety and consumer preference.

**Bleaching agents**

Benzoyl peroxide (BP) has been used for over 50 years as a bleaching agent in flour, whey processing and milk for Italian cheese making. It is used for bleaching of Cheddar cheese whey using at 20 mg/kg BP and holding for an hour at 60-63°C. As benzoyl peroxide is almost totally converted (> 91%) to benzoic acid during cheese making and any remaining traces would further be reduced by processing of whey. Therefore the intake assessment should be made on the additional benzoic acid incorporated in the diet from the use of benzoyl peroxide to bleach whey. Only 15% of the world's cheese production is coloured and hence is subject to use BP. Besides, not all of the coloured whey undergoes bleaching process before drying.

Benzoyl peroxide is colourless, crystalline solid having a faint odour of benzaldehyde. It is insoluble in water, slightly soluble in alcohol, and soluble in chloroform and ether. One g dissolves in 40 ml of carbon disulfide. It melts between 103°C and 106°C with decomposition. Benzoyl peroxide, especially in the dry form, is a dangerous, highly reactive, oxidizing material and has been known to explode spontaneously.

It has been reported that benzoyl peroxide is typically used in the cheese manufacturing at a level of 20 mg/kg to bleach milk used for the production of white Italian cheeses e.g. Asiago fresh, Asiago soft cheese, Asiago medium cheese, Asiago old cheese, Blue cheese, Caciocavallo siciliano cheese, Gorgonzola cheese, Parmesan and Reggiano cheese, Provolone cheese, Romano cheese, Swiss and Emmental cheese. The FDA has affirmed benzoyl peroxide to be GRAS when used as a bleaching agent, following current GMP conditions of use, for the above-mentioned foods.
**Inhibitory salts**

**Nitrates**

In manufacture of less acid type cheese like Edam, Gouda, Swiss, Svecia, etc., inhibitory salts like sodium or potassium nitrate are added in milk to prevent the growth of gas producing organisms such as coliform/aerogenes groups of bacteria which are responsible for "early blowing" defect in cheese and butyric acid bacteria which are responsible for "late blowing" defect in cheese.

Sodium nitrate, when added to milk, does not affect the growth of lactic bacteria or gas forming propionic bacteria desired in manufacture of Swiss type of cheeses. On the other hand, it inhibits the aerogenes group of bacteria. Nitrate in combination with salt (NaCl) in cheese help to control the butyric acid bacteria (i.e. Clostridia) responsible for late blowing defect in cheese. It is not the nitrate itself that is active against clostridia (e.g. *Clostridium tyrobutricum*) but the nitrite that is derived from it. A concentration of 10 to 100 ppm of nitrite or 2 to 5 % of nitrate is sufficient to inhibit growth from spores.

Sodium nitrate (Salt petre) or potassium nitrate (KNO₃) is added at the rate of 20-30 g per 100 litres milk. Lower than this amount may be added when altered/special steps are followed in manufacture of cheese, e.g. bactofugation - 15 g/100 litres. 7.5 g/100 litres is the minimum effective amount when the milk is not heavily contaminated with spores. Bactofugation + lysozyme treatment of milk permits complete elimination of nitrate addition or a very low amount is required (e.g. 2.5 g/100 litres).

Nitrate addition to milk for cheese making may have limitations like (a) production of colour defects in cheese (b) carcinogenic effect (c) larger quantity of nitrate has adverse effect on propionic fermentation (essential for eye formation) and hence effect on eye formation is apparent (d) sometimes bitterness defect may also be found and (e) production of whey having a high concentration of nitrate and nitrite content, especially when concentrated and dried, creates problem in feeding young animals and in human nutrition (requires treatment of whey by ion exchange or electrodialysis).

The inhibition of late blowing by nitrite depends on several factors, viz., (i) the nitrate concentration used, (ii) the treatment of the milk, (iii) the pH of the cheese at 24 hour, (iv) solids/moisture content of cheese, (v) salt content of cheese, (vi) spores (*Clostridium tyrobutricum*) level present in milk and (vii) presence of microorganisms capable of converting nitrate to nitrite and its decomposition.

**Nisin**

The name nisin was assigned by workers at the NIRD, Shinfield, England to the antimicrobial substance produced by Lancefield Group N *Streptococcus lactis*. The joint FAO / WHO Expert Committee on Food Additives defined nisin as the name given to
several closely related polypeptide anti microbial substances produced by strains of
*Streptococcus lactis*. Nisin is a polypeptide having 34 amino acids and its molecule is
in the form of a pentacyclic peptide, containing sulfide bridges contributed by
lanthionine and β-methyl lanthionine. This structure corresponds to a molecular
weight of 3510.

Nisaplin when stored in original unopened containers, is stable for a period of two
years from date of manufacture, when stored dry, away from direct sunlight and at
temperatures in the range of 4 to 25°C. Nisin is acidic in nature and exhibits greatest
stability in acid conditions. For example, pH 2 it is remarkably stable and can
withstand prolonged storage at 2 to 7°C or drastic heating at 121°C without loss of
activity. It is apparent, therefore, that some loss of nisin activity is inevitable when
Nisaplin is used to preserve heat processed foods such as processed cheese.

Nisin (i.e. Nisaplin) has been found to exhibit an inhibitory effect on certain species
and strains of Gram-positive bacteria. It has no effect on true Gram-negative
bacteria, and has no action against yeasts and moulds. The bacteria, which are
completely insensitive to nisin, include *Aerobacter, Alcaligenes, Brucella, Escherichia,
Proteus, Pseudomonas, Salmonella and Shigella*. The bacteria, which exhibit marked
sensitivity to nisin, are certain strains of *Lactobacilli, Streptococci, Micrococi, Spore
forming species of Bacillus and Clostridia*.

**Sorbic acid**

Sorbic acid is a straight chain, α, β – unsaturated trans – trans, 2, 4 – hexa – dienoic
monocarboxylic aliphatic acid and has the molecular formula (C₅H₇COOH)

The carboxyl group of sorbic acid reacts readily and forms salts and esters. The salts
of sorbic acid, especially the potassium salt, are very important in application due to
high solubility in water. The potassium and sodium salts of sorbic acid are easily
soluble in water and act up to a pH of 6.5. The very low water solubility (i.e. 0.16
g/100 ml water at 20°C) is a disadvantage for sorbic acid. Solubility of sorbic acid in
water increases with pH and temperature.

Sorbate has been shown to inhibit growth of yeasts, moulds and many bacteria. Its
activity against bacteria, however, is not as comprehensive as that against yeasts
and moulds. Several workers have amply demonstrated the usefulness of sorbic
acid, as a mould inhibitor in cheeses.

Several theories regarding mechanism of inhibition are there but no single
mechanism can be held responsible for the antimicrobial activity of sorbate. The
possible mechanisms include (1) prevention or delay in growth through a static or
cidal effect either on the spore or the cell, (2) prevention by inhibiting cellular
uptake of substrate molecules such as amino acids, phosphates, organic acids and
the like (3) prevention due to the inhibition of dehydrogenase system and (4)
inhibition of various enzymes systems and their reactions has been implicated as
the mechanism of microbial growth inhibition by sorbate. Some of the enzymes systems reported to be inhibited are (a) Inhibition of certain dehydrogenases, which are involved in the $\beta$-oxidation of fatty acids. (b) Sulphhydryl-containing enzymes (e.g. succinic dehydrogenase, fumerase, aspartase, yeast alcohol dehydrogenase), (c) Inhibition of respiration through its competitive action with acetate on the site of acetyl CoA formation (d) Inhibition of the activity or synthesis of the enzyme catalase and (e) Inhibition of transport of carbohydrates through membrane.

Sorbic acid has been tested and used as an anti-microbial preservative in a wide range of products. Sorbic acid and its potassium salt are the most widely used forms of the compound and are collectively known as sorbates.

Depending up on specific needs sorbic acid or its salts may be used in cheese industry by following a single method or a combination of methods such as direct addition into the product, dipping in a sorbate solution, spraying with a sorbate solution, dusting and incorporation in the wrapping or packaging material. The addition rates in the product may vary from 0.01 to 0.30 % depending on product type and its characteristics.

The sorbates can be incorporated in a binding agent for application to the substrate. A minimum quantity of 3–4 g of calcium sorbate per square meter is necessary to be effective. To be effective the packaging material must be in close contact with the product.

**Natamycin/Pimaricin**

Natamycin/Pimaricin is a fungicide produced by *Streptomyces natalensis*. Natamycin/Pimaricin is tetraene, which in its pure form is obtained in crystals. Its empirical formula is $C_{33}H_{47}NO_{13}$. A trade product 'Delvocid' containing natamycin is a commercial fungicide developed and patented by Gist-broecades NV (Delft - the Netherlands).

Delvocid has no colour, odour or taste. It is non-toxic. The solubility in water is lowest at neutral pH but it increases at low and high pH. At room temperature about 50 mg dissolves in 1 litre of pure water. This low solubility allows it to remain on the surface and therefore it is active at the very places where most moulds and yeasts occur, yet does not affect the fermentation processes essential in certain food products. Thus it is very suitable for the surface treatment of foods. Delvocid is amphoteric. Delvocid is very active against nearly all moulds and yeasts, but not against viruses, bacteria and other microorganisms. It is very effective in extremely small quantities against moulds and yeasts that may occur on food products and cause their deterioration. Since it is not active against bacteria, it does not interfere with the natural ripening process in cheese.

Delvocid Instant is a powder containing 50 % of the active ingredient Natamycin. It can be easily suspended in water, lends itself admirably for use on cheese when no
coating is required and is very suitable for all cheese ripened under film. It can be applied to cheese by means of (i) dipping and (ii) spraying with an aqueous suspension. For certain cheese types it is customary to incorporate the fungicide in the plastic coating.

**Emulsifying salts**

Emulsifying salts are vital ingredients responsible for the characteristic features of processed cheese production. They provide uniform structure during the melting process and also affect the physical, chemical and microbiological quality of the resultant product. A proper understanding of the type and role of emulsifying salts is quite essential for the processed cheese maker, since their appropriate and selective use can improve the product quality and consumer acceptability.

Most commonly used emulsifying salts are classified as citrates and phosphates (Mono phosphates and Poly phosphates as chain, ring or cross linked ultra phosphates).

Phosphates are salts of phosphoric acid; a distinction is drawn between mono and polymeric phosphates. Two types of phosphates are used. (1) Mono phosphates (orthophosphates), e.g. NaH2PO4, Na2HPO4 and Na3PO4 and (2) Condensed polyphosphates: e.g. Poly phosphates, Meta phosphates – rings, e.g. Na3P3O10; Na4P4O12 and Condensed phosphates - rings with chains and branches.

Citrates are salts of citric acid and highly soluble with fairly good protein dissolving power. Cheese produced with citrates shows little tendency to absorb moisture and structure remains firm and heavy. They are preferred in block type cheese. The lack of creaming action deficient bacteriostatic properties and mottling in cheese structure are the main disadvantages of citrates. Tri sodium citrate gives processed cheese its characteristic flavour and has good buffering, complexing and protein dissolving power.

**Role/functions of emulsifying agents**

The ability to sequester calcium is one of the most important functions of emulsifying agents. The principal caseins in cheese (αs1-, αs2-, β) have non-polar, lipophilic C-terminal segments, while the N-terminal regions, which contain calcium phosphate, are hydrophilic. This structure allows the casein molecules to function as emulsifiers (Shimp, 1985). When calcium in the calcium para caseinate complex of natural cheese is removed during processing by the ion-exchange properties of melting salts; insoluble para caseinate is solubilized, usually as Na-caseinate.

The essential role of emulsifying agents in the manufacture of processed cheese is to supplement the emulsifying capability of cheese proteins. This is accomplished by (1) Removal of calcium from the protein system, (2) Peptizing, solubilizing and dispersing the proteins, (3) Hydrating and swelling the proteins (4) Emulsifying the fat and stabilizing the emulsion, (5) Controlling pH and stabilizing it and (6)
Forming an appropriate structure of the product during/after cooling. (Caric and Kalab, 1987)

**Cheese ripening salts**

These salts are sometimes added in more acid curd cheese to reduce acidity (i.e. to increase pH). For example, addition of NaHCO$_3$ and CaCO$_3$ mixture may be done along with salt at the rate of 30 g/kg of curd. Na$_2$HPO$_4$ may also be used in place of carbonate mixture.

**Cheese salt (NaCl)**

Salt (NaCl) addition in curd/cheese is an important step in cheese manufacture as it has a decisive influence on composition of the product, physicochemical state of the water in the product and the quality of ripening. Salting of curd is a traditional and integral part of the manufacture of most cheese varieties.

The amount of salt to be added to cheese depends upon (1) Amount of curd in vat: This depends upon (a) composition of milk used (b) moisture content of the curd (c) losses of constituents up to milling; (2) Salt content desired. It is now well recognized that salt concentration (particularly Salt in moisture, S/M) exercises the strongest influence on cheese quality. Therefore, S/M content should be controlled within certain range (e.g. ~ 5 in Cheddar cheese). Avoid a low (i.e. <1.4 %) or high (i.e. >2.0 %) salt content. Likewise ensuring consistently uniform distribution of salt throughout the cheese block or the blocks from the same vat is essential, but difficult in commercial practice and (3) Condition of the curd: Condition of curd influences salt absorption and its retention, e.g. wet curd retains less salt.

If under salting is done then (a) Cheese breaks down rapidly, (b) Flavour of cheese is abnormal, (c) Cheese will be having Pasty, weak body and (d) Open texture. Over salting would result in (a) Delayed ripening of cheese, (b) Close texture, (c) Cheese is hard, dry and harsh in body and (d) Cracked rind

The cheese salt should be coarse (such salt dissolves slowly, thus better penetration due to longer contact time), free from lumps and extraneous matter, should be dry and be free from impurities like magnesium chloride and calcium chloride.

**References**

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• Product bulletin on natural food colours by the manufacturer – Chr. Hansen's, Laboratorium A/S, Copenhagen, Denmark.
• Product bulletin on ‘Deltocid’ by the manufacturer – Gist – Brocades NV, Delf, Holland.
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• Upadhyay KG (2001) Essentials of Cheese making. D
Cheese making once up on a time was a craft, but now has become science. The traditional cheese making has given way to industrial production, using either various cheesemaking equipments or employing semi automatic to fully automatic cheesemaking lines. In this write up different equipment used in cheese making are explained. However, for more details on the subject, interested readers may refer the references cited at the end of this chapter or refer the equipment manufacturers.

The mechanization of cheese making operations has reduced labour and time of cheese making and increased the utilization of existing equipment. However, it must be accepted with caution and due consideration to various factors, such as the type of cheese and the quality (i.e. appearance, texture, flavour, etc.) requirements to be met, scale of production, losses of solids in whey and regularity of weight for cheeses sold by the unit, degree of mechanization required, labour saving, capital costs involved, etc.

**Cheesemking equipment**

All the standard items of dairy equipment-reception, pipelines, storage tanks, pasteurizing equipment, separators, pumps (for milk), and everything used up to the cheese vat stage, are usually identical with those used for liquid milk plants or manufacturing creameries in general. Suitable types of conveyors, hoop (mould) washers, fork lift trucks may be slightly different from the types normally used in modern liquid milk plants but are basically the same. The specific items of cheese making equipment are (1) Cheese vats, (2) Knives – cutting devices, (3) Stirrers or agitators for milk / whey, (4) Stirrers for curd, (5) Whey removal equipment, (6) Curd washing equipment, (7) Curd cutting/ milling, (8) Curd working and cooling equipment, (9) Moulding and shaping equipment, (10) Salting, (11) Cheese moulding and hooping, (12) Cheese fillers, (13) Conveyors, (14) Cheese press, (15) Brining equipment, (16) Cheese packing equipment and (17) Equipment / facilities for curing of cheese. Other minor equipment used during cheese making is gate strainers, racks, curd shawls, thermometers, etc.

**Cheese vats**

A cheese vat is used to set the milk to obtain coagulum for cheese. This stainless steel vats have replaced the copper or tinned steel vats used until First World War. One type of vat may not be befitting the requirements to manufacture all varieties of cheeses and hence one has to look for specific features in vats for special requirements.
Cheese vats are mainly rectangular in design with a jacket and special H type arrangement in the bottom for uniform distribution of steam and water supply in the jacket. They are open to atmosphere. The capacity may range from about 100 l to 2000 l or more. The outer jacket may be made out of mild steel. The inside of the vat does not have any sharp corners and welded joints must be properly ground and polished. Normally the cheese vat is provided with a jacket drain valve, overflow connection, gate valve to remove whey, a longitudinal central ridge for smooth escape of whey, dial thermometer, valves for steam and well water as well as chilled water supply. Adjustable legs support the vats or else they have tilting facility. In advanced versions of cheese vats there is also facility for mechanical stirring (either by manual swinging or motorized) of curd and whey mixture and curd at the time of salting. Such vats are suitable for many types of cheeses such as cottage, Cheddar, etc. where whey is drained and then curd is dealt manually. To do away with the dead whey pockets in the vat and to facilitate mechanical agitation, the rectangular vats have been modified to have semicircular ends.

The earlier vats are now replaced by either vertical, short horizontal, oval shaped or cylindrical, enclosed vats complete with mechanical cutting and stirring devices. These are used as curd making and scalding vats, the curds and whey being run into draining tables below. Some of the examples of these types of vats are (1) The Alfa Laval OST vat, (2) The Damrow Co. double O vat, (3) The Golden Vale Engineering Co. W cheese vat, (4) The Wincanton Desco Sherborne enclosed vat and (5) The New Zealand Vatmaster vertical enclosed vat. Similar vats, with additions as required by the cheese makers, are marketed by many firms world over, yet the present tendency is towards the horizontal enclosed vats. Some of the vats of this type in use include (1) The Alfa Laval OST 111 horizontal cheese maker, (2) The Alfa Laval Cheddar Tank and (3) The Gadan OS 1000 cheese processing tank. As the firms have incorporated various features as per requirements of their customers a standard vat is unusual.

The vats may have volumetric meters for starter or water addition and CIP system fitted on them. Since the vats are enclosed, automatic control in respect of operations is a feature of these installations, i.e. filling, adding starter, renneting, cutting, stirring, scalding and emptying. Where the bottom of the vat is not made sloping, the vats are provided with tipping gear, pneumatic or hydraulic, to tilt the vat for emptying. The speeds (2-15 rpm) and rotation of the agitators are controlled through an automatic control panel but it is desirable to have manual override of the operations for those occasions when difficulties arise. Certain points which may need consideration are (1) The nature of steam supply and the water supply in respect of scale or rust formation, (2) The nature of water used for rinsing which may leave a dried on deposit on the milk contact surface, (3) The positioning of spray balls, etc. in the CIP system, (4) The electrical /electronic control systems as affected by moisture laden atmospheres, (5) The efficiency of the sampling and
testing devices (6) The efficiency of agitation is important, especially when rennet is added, otherwise a number of different areas of curd firmness will occur in the body of the vat and (7) The nature of the air supply for pneumatically operated valves, thermometer transmitters, pressure controllers, etc. must be such that moisture cannot accumulate in the pipes.

**Cheese knives and cutting devices**

In cheese making the coagulum is cut to expel whey and facilitate further operation. In olden time the coagulum was cut by cutting fork (in UK) whereas today the wire knives (known as American wire knife) are used. The knives come in two styles, horizontal and vertical. In both types, stainless steel wires are brazed on stainless steel rectangular frame support at a specific distance (0.5 to 2.5 cm.). The knives are provided with handle for smooth working. In modern cheese vats, agitators perform the cutting operation by changing the direction of rotation. For very large vats the knives can be fitted on a vertical shaft to achieve cutting.

**Stirrers/agitators**

For thorough mixing of starter culture, additives and rennet in cheese milk as well as for efficient heat transfer to achieve uniform heating of the curd/whey mixture it is essential that the contents of vat be stirred satisfactorily. At farm/small scale level this is possible using an agitator like a rack or plunger made of some metal or wood by moving it to and fro in the vat. For large scale operations either paddle or crockatt type agitators are used. They are made up of metal and consist of a long metal bar attached to a shaft, which is held lengthwise on the vat. The shaft then oscillates through about 60° moving the bar across the vat from side to side.

The cut curd after milling is stirred to facilitate cooling, exhale some odours and permit proper mixing of salt in the cheese. This is usually achieved by employing fork type agitators fitted on a vertical shaft.

**Whey removal**

Depending on the variety of cheese the whey and curd are separated in different methods from the curd-whey mixture. A common practice is to allow the cooked/scalded curd to settle which is then pushed to one end of the vat using a perforated rack. A gate strainer is then inserted in the valve of the vat and whey is drained. The curd at times is also collected in cloth bags (e.g. in ancient Swiss cheese making) and hanged to facilitate further moisture expulsion. In some other varieties the whey-curd mixture is directly delivered in the hoop and handled further (e.g. Gouda cheese). Now a day, the curd-whey moisture is either pumped to a curd-processing vat having a screen at the bottom or is passed through metal screens, which retain curd on them. For soft varieties a special type of curd separators (e.g. Quarg separator) are employed.
Other forms of equipment for whey removal included inverting arrangement, whey absorbing devices and centrifugal separators. A new system known as Table drainer has become a useful piece of equipment following curd maker vat. Typically the drainer is a long rectangular tank with a movable screen belt as a base. One end of the tank has a liquid tight but movable door. In use, the curd/whey mixture is run from vat into the tank. The whey drains through the base screen, leaving the curd level and dry. Some tanks are fitted with plates to press the curd lightly. When the dry curd is moved through the open end of the tank on its screen base it is cut into blocks by a guillotine attachment. The blocks of the curd can be filled into cheese hoops for further pressing, salting, etc.

**Curd washing**

Washing of curd is one of the steps in the manufacture of certain soft varieties of cheeses like Cottage. In such varieties, after draining of the whey the curd is usually washed by spraying wash water. In mechanized system the curd is placed on the perforated belt and passed through a shower of chilled water.

**Curd cutting/milling**

With those varieties of cheeses where maturing of curd takes place before hoping e.g. Cheddar, the curd is divided into small pieces/fragments before it is salted and packed in hoops. The cutting is usually done either by power operated peg mills or by rotating disc cutters. Most of the mechanical equipment employed for manufacture of Cheddar cheese includes automated milling. Both the Alf-O-Matic Mk II and Damrow DMC units are fitted with a curd mill at the end of cheddaring conveyor.

**Curd working and cooling**

For soft cheeses like Quarg cooling is an integral part of manufacturing process. The cooling in such case is obtained by passing cheese using an auger through a horizontal jacketed cylindrical heat exchanger.

**Curd moulding and stretching**

Some varieties, especially Italian, have unusual shapes. It is therefore necessary to have special equipment for mechanical moulding of curd for Italian varieties. The curds after attaining the desired degree of acidity are transferred into a mixing or kneading machine similar to a baker’s dough mixer for further processing. The outlet of such machine may be linked to a screw extruder for Mozzarella type cheese.

**Cheese hoops/moulds**

Traditionally cheeses were not given a specific shape except that of a ball. Subsequently wooden moulds appeared for the purpose until 1900 when they changed from wood to iron and then to tinned steel. Later SS, aluminum or its alloys
became popular for cheese moulds. Plastics of various compositions are now replacing metal for cheese moulds. Attempts to dispense with cloth as liners were aided by the introduction of finely perforated metal sheet. However, the use of plastic of the right type and surface finish for the moulds allows cheese rind to mat without the cloth liners. The shape of the mould may be cylindrical or rectangular. However, the rectangular moulds are becoming more popular due to volume advantage.

The aim of filling curd in mould is to give cheese its typical shape – the closed ring. The shape of mould and lid are so designed that after pressing, the cheeses leave the mould in nearly perfect shape and trimming is no longer necessary. To protect cheese from damage it can be removed from the mould by blowing air or applying vacuum. Electromagnetic vibration sieve or vibration tray for proper removal of whey from curd is used and then mould filling is carried out.

**Cheese pressing**

The very hard, hard and semi hard varieties of cheeses are pressed either in vertical or horizontal presses under normal atmospheric conditions and/or under vacuum to acquire a close texture and good surface. The pressing parameters, i.e. pressure, duration, temperature, etc. give the cheese its final shape, produces a cheese with a firm and smooth surface, and helps to lower its moisture content to the desired level.

Gravity pressing is used mainly for cheeses with high moisture content, but can also be used for hard cheeses. In latter case the pressing takes place at a high temperature, 36-40°C. Great care must be taken during this treatment to ensure that the acid development process is not arrested.

Short pressing for 2–3 h takes place in pneumatic or hydraulic press at 40-50 kPa, after which cheeses are removed from the moulds and cooled quickly. This method is used for cheeses with medium moisture content. A longer period at high pressure is used for Cheddar 100-150 kPa (1-1.5 kg/cm²) for up to 24 h.

The pressure applied is dependent on the type of cheese and it can be achieved using either (1) a spring-loaded or screw mechanism or (2) hydraulic presses or (3) pneumatic systems or (4) vacuum presses. The former type is manually operated and hence the other systems are more common in mechanized cheese factories. One important aspect, which must not be overlooked, is the difference between the ‘airline’ pressure and the ‘actual’ pressure applied to the cheese, the former pressure is normally lower. Since the pressure is defined as mass per unit area, the calculated ‘actual’ pressure on the cheese takes into account (a) the air line pressure, (b) the diameter of the cylinder head and (c) the surface area of the cheese.

In general the cheeses are pressed in individual moulds (i.e. in single or multi row) or in bulk. Different cheese pressing systems available today include: (1) Horizontal
“creeping” or Gang press, (2) Vertical press, (3) Table trolley, conveyor and/or tunnel presses, (4) Rotary press, (5) Vacuum presses and (6) Bulk presses (large hoop or ton press).

**Salting/brining**

Salt can be applied to cheese in four ways: (1) In the whey, (2) In the curd, (3) on the curd and (4) In brine. Of these, methods 2 and 4 are most widely practiced. In small-scale cheese making, the salting is usually performed by hand sprinkling, but for large-scale production it is nearly performed mechanically. In developed system like Alf-O-Matic, the warm dry air transfers the salt from the storage to a salt metering belt. At the end of the belt salt is sucked up into the cyclone and then falls into a rotary valve. Hot air passing through the valve transfers the salt to a stainless steel, oscillating distribution tube to be spread onto cheese curd. The amount of salt added is controlled by a floating sensor that determines the depth of the milled curd, and actuates the salt metering device either to increase or decrease the salt dosage.

Certain cheese varieties e.g. Swiss, Mozzarella are immersed in brine solution. In past wooden, concrete or glazed ceramic tiles brine tanks were used, however, plastic tanks are now being used. The brine tanks may be above floor level or half buried or even below floor level. Automatic filling and emptying conveyors for the cheese are normal in large installations. In some instances cheese is loaded on racks, which are lowered into sunken brine tanks. Large tanks may have brine circulation within tank since these speeds up the operation. Temperature control is also required. For the smaller cheeses portable tanks have been especially developed.

**Packaging**

Cheese packaging can be broadly grouped as (1) wrapping of the cheese for storage and ripening and (2) packaging for consumers. Bulk cheeses are either parafinned or vacuum packed in flexible film. For waxing the cheeses can be lifted by means of suction and half immersed in wax or plastic and then other half can be immersed. With regard to vacuum packaging there are now available vacuum packaging machines, gas-flushing machines, over wrapping machines and vacuum skin packaging machines. Most of the machines are equipped with weighing and labeling facility.

For customers various types of packaging machines are available which can also cut the cheese quickly into convenient customer size without waste and wrap in short time. Vacuum packaging to obtain heat shrink bag or pouch for retail sale is also practiced.

**Miscellaneous items**

Miscellaneous items used during cheese making include: thermometers, gate strainers, cheesecloth, samplers, plungers, etc. The thermometers used in cheese making must be accurate and be housed in a sturdy casing to avoid breakage. This
may be alcohol filled or mercury filled. Coloured-alcohol filled thermometer is preferred because of ease in observation. Floating type thermometers are also available and used. The gate strainers, samplers, plungers, etc. used in cheese making are made out of stainless steel and need no discussion.

**Mechanization and automation**

Cheese is probably the only product, which has exhibited greatest resistance to continuous and mechanized manufacturing owing to various reasons. However, lately it has also yielded to mechanization and automation.

Attempts for the mechanization of cheese making operations started in 19th century. Till 1970 there was not much difference in the terms “mechanization” and “automation”. But today they are considered as different terms.

Mechanized system is a machine in which all or nearly all the stages in cheese making are carried out by machinery instead of manually as in case of traditional cheese making.

Automation is a mechanized system, which is controlled by an instrument fed by a programmer. A continuous system is a process in which milk is fed at one end and is continuously converted into coagulum curd and cheese during passage through the machine.

Today mechanization in almost all steps involved in cheese making is available. Various pretreatments involved in cheese making were already mechanized, now starter production, curd making, cutting, cheddaring, hoping, conveying, tilting, etc. have also been mechanized rather automated. A wide number of mechanized methods are now available to prepare a variety of cheeses on varying levels. Some of the examples are Bell-Siro/cheesemaker, Cheddar master, Lacto-matic, Tebel-Crockatt, Alf-O-Matic-II, the Damrow system, etc.

The mechanized cheese lines used for Cheddar, Mozzarella and Processed cheese and some related equipment are discussed in the presentation.

**References**


FOOD SAFETY AS APPLIED TO FERMENTED FOOD PRODUCTS

G.S. Rajorhia

Introduction

Fermented foods are amply recognized for their superve nutritional value, enhanced sensory properties, functionality, extended shelf-life and numerous value additions and many techno-economic benefits. Fermented foods add a variety of taste and texture to our daily menu depending upon the types of raw materials (cereals, pulses, fruits and vegetables, fish, meat and milk, sugar and molasses, etc.), the kind of starter culture used (yeasts, molds and bacteria) and the extent of fermentation. Cultured and fermented foods are known by different names and nomenclature around the world. With increased scientific evidence of the health benefits and growing awareness among the consumers about the healthy attributes of fermented foods, vast potential exists for the industrial growth of this food sector by promoting primary agricultural production and processing industries. Production of fermented food products using modern science and technologies is expected to increase the income of farmers and their families to boost rural development.

As our knowledge of the role microorganisms play in human nutrition, immune functions and disease resistance increases, the number of fermented products will continue to grow in the market. It should be necessary to ensure that the microbes consumed are alive and active in some fermented products, but metabolites and other fermentation ingredients remain definitely active in other products. Region specific fermented foods are consumed in all parts of India in daily diets, be it Idlis, Dosa and pickles in the south, dhokla, khaman and srikhand in the west, Misti-dahi in the east and dadhi, chhachh and kadi in the north. Fermentation has the potential of enhancing food safety by controlling the growth and multiplication of a number of pathogens in foods. Consumption of cheese in India is growing at a speed not foreseen earlier.

Food Safety and Standards Act, 2006

Historically, in India, multiple laws and regulations prescribed various standards regarding food. However, due to variation in the specifications/standards and the spread of different regulations in various Ministries/Departments of the Government, there were several difficulties in implementation of the regulations. The food industry was facing immense problem as different products were governed by different orders and there was limited scope for innovations and development. The country needed the consolidation of the food laws. Food safety and Standards Act, 2006 is an Act to integrate food laws, to serve as a single reference point of implementation of food regulations and scientific developments of standards. Under
this Act, the Food Safety and Standard Authority of India (FSSAI) has been established for laying down science-based standards for food products and to regulate their manufacture, storage, distribution, sale and important, to ensure availability of safe and wholesome food for human consumption.

Genetically modified foods, organic foods, functional foods, nutraceuticals and proprietary foods shall also be regulated by this Act. Labeling requirements and advertising norms will also be developed in the interest of consumers. There is a provision under FSSA to establish various Scientific Panels on: (a) Food additives, flavourings, processing aids and materials in contact with food; (b) Pesticides and antibiotic residues; (c) Genetically modified organisms and G M foods; (d) Functional foods, nutraceuticals, dietetic products and other similar products; (e) Biological hazards; (f) Contaminants in the food chain (g) labeling and (h) methods of sampling and analysis. These panels have already been constituted.

**Labeling, claims and proprietary foods**

Labeling of food products is an important bridge between the producers and consumers. It is important that necessary information about the food product is made available to the consumers. Information regarding nutrition, shelf-life, weights/measure, food additives, storage conditions, type of food (veg/non veg), etc. is important. Also, if any product makes any health claim the same should be expressed clearly and supported by adequate detail. It is desirable that only guidelines are developed to adopt good labeling procedures and defining of health claims. This will help bring about uniformity, clarity and simplicity in labeling and avoid confusion in implementing the regulations. It is important to prevent misleading health claims which finally create mistrust between the consumer and the industry.

**New product development**

To give the consumer wider options on product variety, product development/innovation is required. Product innovation is a global phenomenon and we need regulation which can channelize the same. Therefore, permission for use of certain food additives needs to be given on safety norms and ADI limits. Further, it is humanly neither possible nor desirable to prefix product formulations. All such food products for which standards are not prescribed are termed as Proprietary Foods. We need to provide adequate opportunity to food processors to develop and produce proprietary foods within the ambit of food safety norms. India is a vast, multi-cultured society having immensely rich varieties of cuisines. We need to encourage our traditional products. Perhaps that would be India’s strength in times to come.
Food additives

An important aspect of food standards relates to food additives. The type of additives to be used and their quantities are of importance. Detailed studies have been carried out in the world regarding the use of food additives and well developed standards are also available internationally e.g. Food Acts under USFDA, EU, ANZAF etc. We need to harmonize our standards with them and extend the scope of use of different food additives in our products. We shall require evaluating the use of new food additives in a scientific manner.

Besides the permissible limits of food additives, we also need to focus on the quality and their production process. Risk assessment studies will be required for adopting new food additives. Appropriate labeling of certain food additives is important, particularly when the product is meant for infants or for special application. This will be a major scientific aspect to be examined by the Food Authority.

Significance of food safety

Food safety describes the ways and means of handling, preparation, storage and distribution of food to prevent food borne illness by ensuring absence or the lowest safe levels of contaminants. It is generally agreed that food will be safe to eat and cause no harm to consumers when prepared and eaten according to its intended use. Food safety hazards can enter at any stage of food chain.

Food safety occupies a centre stage in the food chain because: new products are coming to the market at a fast pace; new processing methods and equipment are being developed; world markets are opening up and pattern of consumption changing; information about emerging pathogens building up; World Trade Organization agreements for safe food business; formulation of food safety guidelines and standards (ISO 22000; BRC, SQF, Global GAP, HACCP, GAHP, GMP) backed by international consumers and harmonization of requirements for systematically managing safety in food supply chain. All the food safety management systems are based on hazard analysis and critical control points. The international food safety standards specify the requirements to enable an organization to plan, implement, operate, maintain and update a food safety system aimed at providing safe products for consumers and to demonstrate compliance with applicable statutory and regulatory food safety requirements of the countries.

The FSMS is being adopted by many food business operators to analyze and establish measures to prevent possible food hazards in the foods. Adoption of FSMS is imperative to reach higher product standards and develop confidence in consumers. The Food Safety Authority has already framed licensing and registration regulations for food business operators which include mandatory norms of food safety systems. The biological, chemical, physical and environmental risks particularly those that are life threatening should be eliminated or effectively reduced.
Commonly known food contaminants

The known sources of food contamination include pathogens, harmful chemicals, heavy metals, radionuclides, pesticides, preservatives, colouring agents, additives, trans fats, genetically modified foods, allergens, adulterants, etc. According to the Centre of Disease Control and Prevention WHO, in U.S. alone, about 76 million cases of food borne illness are reported annually leading to 325000 hospitalization cases and 5000 deaths. Food borne illness of microbial pathogens biotoxins and chemical contaminants in food represent a serious threat to the health of millions of people in the world and more so in the developing countries where awareness about hygiene, sanitation and food safety is lacking. The use of modern antibiotics on British farms has risen gramatically in the past decade fueling the development of resistant organisms and weakening the power of human medicine to cure diseases. The Health Protection Agency of the U.K. has published a report showing a sharp rise in bacteria resistant to carpenems, a new and strong type of antibiotic of global public health concern.

Food safety risks in fermented products

The fermentation products require a whole spectrum of materials. New developments in fermentation technologies such as microencapsulation, prebiotics, preservation methods and the different types of activation, inoculation, propagation, processing, packaging and storage methods either singly or in combination may lead to serious consequences in respect of food safety especially while handling large volumes of production for network distribution. Use of new types of microorganisms and those developed by molecular engineering would require extensive risk analysis studies to ensure safety. Fermented products are prone to rapid spoilage at high humidity and temperatures unless properly kept in refrigerated condition during storage, transport and distribution.

The food safety risks associated with fermented foods are high primarily because many of them receive very little or no thermal processing before consumption. The hazards may be classified as intrinsic, extrinsic and biogenic.

Intrinsic hazards: are associated with the properties of the food itself. The additives such as the acidulants, colours, flavours and emulsifiers added and the natural toxins e.g. cyanogenic glycosides, alkaloids, phytic acids, etc., may contribute to hazards.

Extrinsic hazards: may be posed by the chemical, contaminants like pesticide residues, heavy metals and infectious pathogens (Salmonella, Shigella, E. Coli., Viruses, protozoa) inadvertantly finding way into the product. Incidence of foreign objects in dairy foods like elastic bands, thread, gaskets, hairs, etc. is reported.

Biogenic and processing related hazards: generated by microbial contaminants (nitrosamines, biogenic amines, bacterial toxins, mycotoxins) and those associated
with processing. The factors which influence fermentation are sometimes very difficult to control particularly when processing is carried out under household conditions or small scale operations. Organized scale production of fermented foods is, therefore, recommended. The major failure in achieving optimized fermentation can be attributed to lack of monitoring of incoming raw materials and lack of hygiene. The lactic fermentation cannot be relied as a method for reducing or eliminating food borne illness. Enteropathogens show some pattern of acid resistance surviving fermentation processes. Yoghurt and fermented meat products have been reported to carry enterohaemorrhagic E. Coli infection. Simian rotavirus has been shown to survive high levels of acidity in model fermented foods. The cysts of certain parasites (Crystosporidium, G. Lamblia and Trematodes) often show resistance to adverse conditions. There is no evidence that bacterial toxins can be degraded by fermentation. Lactic acid fermentation exerts a limited effect on anti-nutritional factors such as protease inhibitor and lectins.

**Safety of lactic acid bacteria (LAB)**

The LAB are generally considered to be non toxic and non pathogenic. However, some species of LAB can produce Biogenic Amines (BA). BAs are toxic nitrogenous compounds, mainly formed by decarboxylation of amino acids. BAs can accumulate in high concentration in fermented foods which may cause toxicogenic manifestations. There is no legislation with regard to the occurrence limits of BAs in foods. It is important to estimate the likelihood of their presence and to prevent the accumulation in food products. Optimization of processing steps during the preparation of fermented foods is essential to avoid the generation of BAs and other toxic materials such as ethyl carbamate during fermentation.

**Assessing the starter culture safety**

The selection of proper starter cultures with a long history of safe use is recommended. Strains that belong to species for which no pathogenicity is reported may be considered for use. Strains carrying transferable antibiotic resistance genes should not be used. Strains not described taxonomically (DNA-DNA hybridization, RNA sequencing) should be avoided. Assessment of active and sub acute toxicity of ingesting extremely large amounts of probiotic strains should be carried out. The effects of the strains on the health of human micro flora human intestines should be assessed. Epidemiological and post marketing surveillance is required to confirm nontoxicity of bacterial strains in fermented foods. The safety of starter cultures is generally assessed by the characterization of genes, species and strains. It will be desirable to study the intrinsic properties of each species and the potential virulence factors. Besides study on adherence, invasion potential and the pharmacokinetics of strains, the interactions between them and intestinal microflora of the host should be conducted to generate data for consideration of legislative bodies.
Conclusions

At present, production of fermented food products is carried out at tiny to small scales without any controls on raw materials, processing operations and hygienic conditions. Modern consumers now demand quality products with assurance of safety. In order to ensure the supply of high grade products and to allow domestic producers to compete successfully with the imported products, compliance to international food safety standards poses a tremendous challenge. Application of standard technologies involving raw material procurement, controlled operations, management of hygienic and sanitary practices and regulated compliances to FSMS are some of the requirements for modernizing fermented food industries. India needs to address the issue of health claims for fermented foods supported by sound scientific data. It is unlikely that any fermented food product will ever carry a disease prevention claim, because the regulations make a clear distinction between foods and drugs. In future, consumers will find more food choices that are good for their health and wellness and also be safe. Manufacturers of fermented foods must get ready for facing the changes.

Reference

Introduction
The heart of a fermentation process is the fermenter. It is a container in which is maintained an environment favorable to the operation of a desired biological process. Historically, the main tool for fermentation laboratory was the shake flask or flat bed bottle. The next stage was the introduction of glass vessel with a stirrer, and this was followed more recently by stainless steel vessels in various sizes and forms. In recent years, microbiologists and engineers have developed several sophisticated mini-fermenters capable of specializing in different types of fermentation.

The environment maintained in a fermenter is a combination of several favorable parameters considered essential for biological process. The biological environment is considered favorable to a biological process when only desirable organisms, contributing to process are present and undesirable, destructive, non-productive are excluded. The chemical environment involves the composition of the microbial growth medium, which should contain the desired concentration of substrate or microbial nutrients free from inhibitory substances. The physical environment refers principally to the temperature of the system and maintaining the uniform conditions throughout the fermenter which involve mixing, agitation and foam control. For maintaining a favorable environment, the environmental parameters are not necessarily held constant with time. A fermentation process passes through different phases of growth which may have different environmental optima to favour the growth. The design characteristics of a fermentation system should thus meet the objective of operation and its applicability.

Principal categories of fermenters
Several different types of vessels are used for large scale biological processes and their degree of sophistication in design, construction and operation is determined by the sensitivity of the process to the environment maintained in the vessel. In the simplest case, the fermenter is a vessel in which reagents, substrates and organisms are brought in contact with provision for their addition or removal. The fermenters can be classed into two broad groups, the liquid fermentation and the solid state / substrate fermentation.

a) Liquid fermenters
Mostly industrial fermentations are carried out in liquid medium using bioreactor system, which range from simple stirred tank or non-stirred containers to complex,
b) Solid state/substrate fermentation

There are many biotechnological processes that involve the growth of microorganisms on solid and insoluble substrates in the absence of free water. The bioreactor design for solid state fermenters is simpler than the liquid fermenters. Such systems can be without mixing, with occasional mixing or with continuous mixing. The use of solid state fermenters is limited to those cultures that can grow comfortably at low moisture levels. These fermenters have advantages of using simple media with cheaper, natural and abundantly available components. Low moisture content required for the process gives economy of bioreactor space, low effluent treatment, less microbial contamination, often no need of sterilization and easier downstream processing. Aeration requirement can be met by simple gas diffusion or by aerating intermittently. Two major advantages of this fermentation method are high yields of products and low energy expenditure compared to stirred tank bioreactor.

Fermenter configurations

There are numerous fermenter types. These include stirred tank reactors, air- lift fermenters, tower fermenters, fluidized bed reactors and rotating disc fermenters. Each of these configurations of fermenters may offer advantages in certain applications.

a) Stirred tanks

Basically stirred tank fermenter consists of a cylindrical tube with a top-driven or bottom driven agitator. The stirred tank with a top drive assembly is the most commonly used fermenter because of its ease of operation, neat design, reliability and robustness. For smaller mini-fermenters borosilicate glass is used as the cylindrical tank and a top plate of stainless steel clamped on it. A motor is fixed above the top plate and is attached to the shaft. The vessel, medium and probes are usually sterilized together, minimizing the number of aseptic operations required. The glass vessel can be protected by a removable stainless steel mesh or jacket. The stirred tanks are available in stainless steel also. They are more expensive than the glass vessels but they are more robust, reliable, and designed to last a lifetime.

b) Air-lift fermenters

In all aerobic fermentations, air is an essential requirement. Air-lift fermenters have no mechanical agitation system but utilize the air circulating within the fermenter to bring about the mixing of the medium. This is a gentle kind of mixing and is ideal for plant and animal cell cultures as there is no shearing action because of absence of mechanical mixer or agitator. The air-lift fermentation is based on the difference in specific weight between the air-enriched volume and low-air volume. As the
fermenter is aerated, the lower density broth (air-enriched medium) creates an upward thrust which results in the circulation of the broth. The type of circulation depends upon the vessel configuration. The basic design for a laboratory scale airlift fermenter is an outer glass hollow tube with an inner stainless tube. A tubular loop type fermenter is designed to increase the volume of the fermentation while maintaining the residence time.

**c) Tower fermenters**

These types of fermenters are designed for continuous yeast fermentation process. Continuous brewing of beer or lager can be carried out successfully in a tower fermenter. The design of these fermenters is simple and they are less expensive than the conventional stirred tank fermenters. Also due to lack of any complex mechanical agitation system they are easier to construct.

**d) Fixed-bed fermenters**

There are several systems developed with a tubular packed bed reactor for laboratory scale applications. The main problem associated with these reactors is getting the fixed bed fully aerated. If air is restricted then anaerobic microorganisms take over. The type of fermentation in fixed beds is a heterogeneous reaction, whereas it is homogeneous in stirred tank fermentation. The fixed bed reactors are widely used in waste water treatment plants.

**e) Fluidized-bed fermenters**

These fermenters are hollow chambers in which dense particles containing a microbial film or microbial mass is recycled. One of the oldest fermentations known to man, vinegar fermentation utilizes this principle.

**f) Rotating-disc fermenters**

They have circular discs which rotate through the medium at slow speed. The microorganisms adhere to the discs and the microbial film is exposed to both the nutrient solution and the air. These types of reactors are being used for waste water treatment.

**Instrumentation and process control**

Increasingly complex control systems are finding application in commercial fermenters for maximizing process productivity and batch-operating-cycle reproducibility. The limiting factor in the possible sophistication of the control system is the limited availability of steam-sterilizable sensors. Various parameters of interest in the process of fermentation could be grouped into two categories, the physical parameters and the chemical parameters.
a) Physical parameters

The physical parameters for which suitable instrumentation exists today include temperature, flow, pressure, agitator shaft speed and power, foam and liquid level.

i) Temperature: Fermentations are run either in the mesophile range (20-45°C) or thermophile (> 45°C) range. The optimum temperature must be chosen to achieve maximum growth on one hand and optimal product formation on the other hand. In some fermentation, higher temperatures are used to obtain increased growth of culture and then the temperature is decreased at the onset of the idiophase. The temperature programming of the process facilitates the optimal temperature policy and corresponding growth and production profiles of product manufactured by fermentation. Temperature is usually measured with a Pt-10012 probe and is controlled with the heat exchange facilities. Because sterilization of the culture medium and removal of heat are vital for successful operation, the fermenter generally has an external cooling jacket through which steam or cooling water can be run. For very large fermenters, insufficient heat transfer occurs through the jacket, and so the internal coils must be provided for circulation of steam or cooling water.

ii) Pressure: Hydrostatic pressure influences the solubility of O₂ and CO₂ in the nutrient solution, especially in the large fermenters. Usually in the fermenters an over pressure of 0.2-0.5 Bar is used in order to minimize the risk of contamination. The elastic deformation sensor elements, such as, diaphragms have employed to sense and control this variable.

iii) Flow: The peristaltic pumps are used for supplying different fluids to the fermenter. The rate of aeration is adjusted to the amount of O₂ required and is usually maintained at 0.25-1.0 vvm (air volume / liquid volume / min). The flow of different fluids is made to pass through motorized valves controlled by the pO₂ regulator.

iv) Agitation: The installation of a continuous drive system is desirable in industrial fermenters in order to be able to precisely adjust the stirring rate to the process. The speed of the impeller varies between 50 to 450 rpm depending upon the fermenter size and is controlled with the help of a dynodrive. The agitator assembly is designed to meet the mixing and aeration requirements.

v) Foam control: Foams are produced in any aerated and agitated culture vessel. Mechanical foam breakers or the addition of chemical antifoam agents such as corn oil, cottonseed oil are the foam controlling methods. Antifoam agent can be directly added to the medium before sterilization or to the fermentation system through a feed line.
b) Chemical parameters

The chemical parameters which can be monitored continuously are limited; only the broth pH, redox, NAD level, dissolved O₂ and CO₂ concentrations and exit gas composition permit direct measurement. Other chemical variables such as RNA and ATP concentrations are also of potential importance, their measurement require increased sophistication and are not employed at industrial level. Of all those mentioned now, pH measurement and control is by far the most common. Modern practice is to control metering of an acid or base into the fermenter to maintain the desired pH. During the course of operation often pH falls due to depletion of an ammonia nitrogen source. Use of added ammonia as the controlling base then serves the twofold purpose to maintain the desired pH and preventing nitrogen limitation of the culture. Monitoring of dissolved oxygen and controlling of air flow rate allows controlling dissolved oxygen concentration as an independent fermentation parameter.

Process control got a boost with the development of biosensors. These are basically the enzyme electrodes and currently hold about 90 per cent of the market, mainly for detecting substrates and metabolites such as glucose, lactate, ATP, ethanol, etc. Recently new techniques have been developed such as Near Infra Red (NIR) spectroscopy analysis, fibre optical methods Flow Injection Analysis (FLA) sensors based on conductivity modifications of a polymer which reacts to vapor. It is possible to measure on-line directly in a bioreactor the concentration of biomass by means of optical density (OD) or by acoustic resonance densitometry (ARD). But very often, problems are encountered with these sensors with regard to reproducibility, drift, response time and sterility.

c) Process control

Fermentation monitoring and control is generally a three-stage process: the first step involves acquisition of data through in-line, on-line or off-line measurements, in the second step these informations are analyzed and processed, and finally in the third step this processed information is used to control the process in the desired manner. Reliable and accurate monitoring of a process is of great importance in an automated control strategy. Due to slow fermentation process dynamics, in many cases the manual process control is the easier choice. Based upon the data acquired through monitoring devices, process variables are identified. Variables may be the directly monitored ones or they may be the derived ones, e.g. oxygen uptake rate, carbon dioxide evolution rate, respiratory quotient etc.

The measuring devices or the sensors sense the value of a process variable. Indicators give a read-out of the variable and recorders log a hard copy of it. The automatic controller compares the measured value of the variable with a prefixed set-point value, computes the error and gives out an error signal. The error signal then actuates the final control element to produce the desired result. This set of
sensor, indicator, recorder, controller and the final control element constitute a
control loop. The most effective control loop is feedback closed control loop and the
common control algorithm being used in the bioprocess industry is PID
(Proportional-Integral-Derivative) control algorithm. It is able to give response
based on size of error (proportional), length of time the error is present (integral)
and the rate of change of error (derivative). Any combination of the three
components can be used depending upon the bioprocess requirement.

d) The software

A microcomputer could be coupled to the reactor to automatically pilot the data
acquisition during the different phases of the process. Different softwares have been
developed to facilitate the repetitive calculations. The user can choose the values of
the set points, the parameters of each regulation loop, the frequency of data
acquisition, the scale on the coordinates axes for the graphic etc. During the
different phases of a process, all the acquisitions are on-line displayed. It is also
possible to display the flow sheet of each reactor and the working of the regulators.
The software also allows checking different parts of the bioreactor equipments.

Downstream processing

The various stages of processing that occur after the completion of the fermentation
or bioconversion stage, including separation, purification, and packaging of the
product.

Stages in downstream processing

Stage 1: Removal of insoluble's- capture of the product as a solute in a particulate-
free liquid For e.g. separation of cells, cell debris or other particulate matter from
fermentation broth. The methods employed are:

a. Filtration: Used for the separation of solids from fluids (liquids or gases)
by interposing a medium to fluid flow through which the fluid can pass,
but the solids in the fluid are retained.

b. Centrifugation: Use of the centrifugal force for the separation of
mixtures. More-dense components migrate away from the axis of the
centrifuge less-dense components migrate towards the axis.

c. Flocculation: Process where a solute comes out of solution in the form of
flocs or flakes. Particles finer than 0.1 µm in water remain continuously in
motion due to electrostatic charge which causes them to repel each other.
Once their electrostatic charge is neutralized (use of coagulant) the finer
particles start to collide and combine together. These larger and heavier
particles are called flocs.

Stage 2: Product isolation- is the removal of those components whose properties
vary markedly from that of the desired product. For most products, water is the
chief impurity and isolation steps are designed to remove most of it, reducing the
volume of material to be handled and concentrating the product. Solvent extraction,
adsorption, ultrafiltration, and precipitation are some of the unit operations involved.

**Stage 3: Product purification** - is done to separate those contaminants that resemble the product very closely in physical and chemical properties. Consequently steps in this stage are expensive to carry out and require sensitive and sophisticated equipment. This stage contributes a significant fraction of the entire downstream processing expenditure. Examples of operations include affinity, size exclusion, reversed phase chromatography, crystallization and fractional precipitation.

**Stage 4: Product polishing** - describes the final processing steps which end with packaging of the product in a form that is stable, easily transportable and convenient. Crystallization, desiccation, lyophilization and spray drying are typical unit operations. Depending on the product and its intended use, polishing may also include operations to sterilize the product and remove or deactivate trace contaminants which might compromise product safety.

**References**

The bioactive peptides which are suggested as an aid in maintaining good health can be ingested as naturally occurring component of food. Therefore these peptides of food origin could be delivered to consumer in conventional foods, dietary supplements, functional foods or health foods. Milk proteins are the main source of biologically active peptides. Many bioactivities in milk are encrypted within the primary structure of milk proteins, requiring proteolysis for their release from precursors. Proteolysis may release these bioactive peptides during gastrointestinal transit or during milk processing. Nowadays the peptides liberated from food proteins during fermentation have gained special interest because they may also influence numerous physiological responses in the organism. Cheese is a major fermented dairy product in which the proteolysis of milk proteins leads to generation of numerous peptides. These peptides not only contribute toward the flavour, taste and texture of cheese but posses a number of biological activities such as angiotensin I converting enzyme inhibitor, opioid, antimicrobial, immuno-modulatory and antioxidant activities. Indigenous protease in milk or residual milk clotting enzyme and bacterial starter cultures are responsible for degradation of caseins during ripening. Therefore the cheese not only considered as a source of vitamins, minerals and high quality proteins but also a potential source of the bioactive peptides.

**Release of bioactive peptides during cheese ripening**

Technologies for the production of milk proteins derived bioactive peptides. The sequences of the bioactive peptides mentioned above are contained within the intact milk proteins and must be released from the proteins by specific enzymatic hydrolysis to extend their health effects. In principle, there are two approaches for releasing peptides from intact milk proteins. One approach is to subject isolated milk protein preparation to hydrolysis in vitro by one or a combination of enzymes containing a great number of peptides among them the bioactive peptides. The hydrolysates or the hydrolysates enriched in particular peptides may be applied in the manufacture of other food products, to provide them with the desired bioactivity. The technological challenges thus lie in the production of milk protein hydrolysates with a high concentration of peptides with a specific bioactivity and with a functionality that makes them suitable as ingredients in other foods, including dairy foods. The benefits of the fermented dairy products in the diet are well accepted and the science of food and nutrition has evolved towards ‘nutrition for optimal health’. The central role of microorganisms in fermentation, especially...
Lactic acid bacteria (LAB) is now widely acknowledged, and it is accepted that these microorganisms can exert beneficial effects through two mechanisms: Direct effects or indirect effects during fermentation where these microbes act as cell factories for the generation of secondary metabolites with health-promoting properties. Among the latter the most important components in fermented milk are bioactive peptides released from milk proteins by members of the genera Lactobacillus, Bifidobacterium and other LAB.

Lactic acid bacteria (LAB) have a very long history of use in the manufacturing processes of fermented foods and a great deal of effort was made to investigate and manipulate the role of LAB in these processes. For the most extensively studied LAB, *Lactococcus lactis*, a model for casein proteolysis, transport, peptidolysis, and regulation thereof is now established. In particular, *Streptococcus thermophilus*, *L. lactis*, *Lactobacillus helveticus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus* (hereafter *L. bulgaricus*) are widely used dairy starters and are of major economic importance. In the milk fermentation processes, the proteolytic system of LAB plays the key role because it enables these bacteria to grow in milk, thereby ensuring successful fermentation. LAB are fastidious microorganisms that require an exogenous source of amino acids or peptides, which are provided by the proteolysis of casein, the most abundant protein in milk and the main source of amino acids. In general, the exploitation of casein by LAB is initiated by a cell-envelope proteinase (CEP) that degrades the protein into oligopeptides that are subsequently taken up by the cells via specific peptide transport systems for further degradation into shorter peptides and amino acids by a concerted action of various intracellular peptidases. While many LAB strains contain CEP, several of these strains, i.e., nonstarter LAB, do not, thus, they rely on starter LAB for the production of peptides and amino acids. These pathways are also of industrial importance because in addition to allowing growth, peptides, amino acids, and their derivatives are also known to contribute to the formation of texture and flavor of the fermented milk products. Therefore, a number of studies were undertaken to unravel the pathways underlying this industrially relevant trait and excellent reviews have covered these aspects of the most extensively utilized dairy starters (Savijoki, 2006). Besides the importance of the proteolytic/peptidolytic enzymes of LAB to organoleptic properties of the final milk product, certain LAB strains are known to contribute to the liberation of bioactive peptides that are thought to promote health beyond the basic nutrition (Pihlanto and Korhonen, 2006). In this respect, the casein molecules of milk are of particular interest because they are known to harbor bioactive peptides that are latent until released by proteolysis. To date, LAB ascribed with such activity includes strains like *L. helveticus* CP790, *L. rhamnosus*GG, *L. bulgaricus* SS1, and *L. lactis* subsp. *cremoris* FT4 (Flavio, 2009). Several reports have indicated that bioactive peptides, besides existing in naturally ripened cheese and other fermented products, may also be produced in vivo after the intake of milk proteins.
Manufacturing of such peptides on industrial scale for use as dietary supplements and pharmaceutical preparations is currently receiving increased interest (Korhonen, 2009).

So the other approach is to exploit the proteolytic system of lactic acid bacteria to partially digest the caseins and whey proteins during the manufacture of dairy products, like fermented milk and cheeses. The technological challenges, thus, lie in the manufacture of fermented dairy products with a high concentration of particular bioactive peptides or their precursors, which upon digestion in the gastrointestinal tract would give rise to the bioactive peptides.

The production of bioactive peptides in dairy products is an appealing approach, since this confers an additional positive health effect to dairy product possessing already a health image and having a long history of safe production. During the fermentation of milk and maturation of cheese, the major milk proteins are degraded into a great number of peptides due to the action of indigenous milk enzymes (mainly plasmin), added coagulants and microbial enzymes (especially from starter and non-starter lactic acid bacteria, LAB). Similarly, several bioactive peptides with different biological activity have been found in cheeses and they may be due to an intense but not excessive proteolysis (Fabio et al. 2004). A wide range of activities has been described in cheeses (Table 1) including ACE inhibitory properties, antimicrobial and opioid and antioxidant activities, enhancement of mineral absorption/bioavailability and immuno-modulatory effects. Moreover some peptides are multifunctional and can exert more than one of the effects mentioned (Meisel 2004).

**Ace inhibitory peptides**

Ong et al. (2007) developed cheddar cheese manufactured with starter lactococci and *Lactobacillus casei*. They found that the IC₅₀ (concentrations of ACE inhibitors needed to inhibit 50% of ACE activity) was the lowest after 24 weeks of ripening in the probiotic cheeses (0.23–0.25 mg·mL⁻¹) compared to 36 weeks for cheeses without any probiotic (0.28 mg·mL⁻¹). Various ACE-inhibitory peptides corresponding to the αs1-casein N-terminal peptides [(f 1–6), (f 1–7), (f 1–9), (f 24–32) and (f 102–110)] and β-casein N-terminal peptides [(f 47–52) and (f 193–209)] were found. There results concur with Meisel (1997) that ACE inhibition in Cheddar cheese was dependent on proteolysis to a certain extent. Meisel et al. (1997) reported the presence of ACE-inhibitory peptides of low molecular mass in several ripened cheeses. The ACE-inhibitory activity increases as proteolysis develops, but the ACE-inhibition index decreases when the proteolysis during cheese maturation exceeds a certain level (the ACE-inhibitory activity detected in medium-aged Gouda was about double that of the long-ripened Gouda). Saito et al. (2000) investigated the antihypertensive activity of peptide fractions prepared from Emmental, Blue, Edam, Gouda and Havarti in spontaneously hypertensive rats (SHR). A water-soluble
Antimicrobial peptides activity is usually expressed by the disintegration of cell membrane, whereby the lipid bilayer of the cell membrane is the principle target. Interaction between the antimicrobial peptide and the cell membrane is an
important requirement for antimicrobial activity. The activity of antibacterial peptides is defined as a membrane-lytic activity, where they tend to assemble to form channels, with specificity for prokaryotic cell membranes. Many peptides have $\alpha$-helical structures, are cationic, and amphipathic, but there are also hydrophobic $\alpha$-helical peptides that possess antimicrobial activity (Epand and Vogel, 1999). Overall, the majority of bioactive peptides including antimicrobial have been identified in milk, milk hydrolysates and fermented milks (Meisel, 2001 and Gobbetti et al. 2004). Just a few reports have considered the potential of cheeses containing antibacterial peptides (Rizzello et al. 2005).

Water-soluble extracts of nine varieties of Italian cheeses’ that differed mainly for type of cheese milk, starter, technology, and time of ripening were fractionated by reversed-phase fast protein liquid chromatography, and the antimicrobial activity of each fraction was assayed towards *Lactobacillus sakei* A15 by well-diffusion assay. Parmigiano Reggiano, Fossa, and Gorgonzola water-soluble extracts did not show antibacterial peptides. Fractions of Pecorino Romano, Canestrato Pugliese, Crescenza, and Caprino del Piemonte contained a mixture of peptides with a high degree of homology. Pasta filata cheeses (Caciocavallo and Mozzarella) also had antibacterial peptides. Peptides showed high levels of homology with N-terminal, C-terminal, or whole fragments of well known antimicrobial or multifunctional peptides reported in the literature: $\alpha s_1$-casokinin (e.g., sheep $\alpha s_1$-casein f22–30 of Pecorino Romano and cow $\alpha s_1$- casein f24–33 of Canestrato Pugliese); isracidin (e.g., sheep $\alpha s_1$- casein f10–21 of Pecorino Romano); kappacin and casoplatelin (e.g., cow $\kappa$- casein f106–115 of Canestrato Pugliese and Crescenza); and $\beta$-casomorphin-11 (e.g., goat $\beta$-CN f60–68 of Caprino del Piemonte). Most of the water soluble fractions had a large spectrum of inhibition (minimal inhibitory concentration of 20 to 200$\mu$g/mL) toward gram-positive and gram-negative bacterial species, including potentially pathogenic bacteria of clinical interest (Rizzello et al, 2005). Ryhanen et al. 2001 showed that the ACE inhibition activity of a new type of identified peptides showed high level of homology with N-, C- terminal or whole fragments of well known antimicrobial sequences reported in literature (Harcolka and Leher, 1998). Prichad et al. (2010) reported that three Australian commercial cheddar cheeses have shown high antimicrobial activities.

**Opioid peptides**

Opioid peptides derived from food proteins have affinities to bind to opiate receptors and express similar opiate activity, which in turn can be reversed by an opioid antagonist, such as naloxone. Naloxone crosses the blood brain barrier and blocks opioid activity, thus being a useful tool to determine specific effects of agonist opioid peptides. Specific organ tissues that exhibit opioid activity includes the spinal cord, adrenal gland and the digestive tract, via both $\delta$ and $\mu$ receptors and the pituitary gland and hypothalamus through the $\mu$, $\delta$ and $\epsilon$ receptors. The $\mu$ type
receptors mediate primarily neuroendocrine function and relate to pain sensation and analgesia. The δ-type receptor is associated with emotions and reward behavior. Other responses of opioid peptides reported include, stress response, analgesia, sedation, dullness, respiratory depression, hypotension, changes of body temperature, as well as an influence on satiation, decline of gastric secretion and changes in sexual behavior.

Muehlenkamp and Warthesen (1996) found either no β-casomorphins in commercial cheese products or the relevant concentration in the cheese extract was below 2 mg/ml. They further noted that the enzymatic degradation of β-casomorphins was influenced by a combination of pH and salt concentration at the cheese ripening temperature. Therefore, if formed in cheese, β-casomorphins may be degraded under conditions similar to Cheddar cheese-ripening. Precursors of β-casomorphins, on the other hand, have been identified in Parmesan cheese. Matar and Goulet (2003) detected β-casomorphin-4 in milk fermented with L. helveticus L89 deficient in X-prolyl-dipeptidyl-amidopeptidase.

**Immunomodulating peptides**

Bioactive peptides derived from cheeses and fermented foods can enhance immune cell functions, measured as lymphocyte proliferation, antibody synthesis natural killer (NK) cell activity and cytokine regulation. Moreover, immunomodulatory peptides might reduce allergic reactions in atopic humans and enhance mucosal immunity in the gastrointestinal tract (Korhonen and Pihlanto, 2003). The peptides released during milk fermentation with lactic acid bacteria, have been found to modulate the proliferation of human lymphocytes, to down-regulate the production of certain cytokines and to stimulate the phagocytic activities of macrophages. The protective effect of a casein-derived immuno-peptide on resistance to microbial infection by Klebsiella pneumoniae has been demonstrated in mice. Gagnaire et al. 2001 identified 91 peptides from emmental cheese, most of them arose from αs1-(51) and β-caseins (28), and a few arose from αs2- (9) and κ-caseins, 28 of which showed various bioactivities in vitro, e.g. mineral-carrying, antimicrobial, antihypertensive and immunostimulatory activities. β-Casein f193-209 contains sequences of a double-function bioactive peptide that inhibits ACE and is also immunomodulatory (Meisel and Bockelmann, 1999). This C terminal fragment is released preferentially by chymosin and lactococcal CEP, inhibit ACE and lactococcal. Moreover this peptide is bitter or contains sequence of bitter peptides (Gobbetti et al. 2002).

**Antioxidant peptides**

Antioxidant properties that prevent enzymatic (lipoxygenase) and non-enzymatic peroxidation of essential fatty acids have also been found in peptides derived from milk proteins. Most of the peptides identified are encrypted in the sequence of αs1-
casein (Fitzgerald and Murray, 2006). The addition of a leucine or proline residue to the N-terminus of a His-His dipeptide, for example, can enhance antioxidant activity and facilitate further synergy with non-peptide antioxidants like BHT or BHA (Butylated Hydroxianisole or Toluene) (Kitts and Weiler, 2003). Milk fermentation has been described as a strategy to release antioxidative peptides from caseins. Lactic acid bacteria are able to degrade the superoxide anion and hydrogen peroxide (Korpela et al. 1997; Kullisaar et al. 2002). The antioxidant activity was moderate in commercial fermented milks from Europe. Histidine and proline have been described as the most important residues in the lipoprotein peroxidation-inhibitory activity of peptides. Seven of the eight peptides identified in highest antioxidative fraction contained at least one proline residue, and six of them had more than two residues of proline. The high content of proline peptides could determine the antioxidant activity found in this fraction. Tyrosine and tryptophan also showed ABTS•+ radical-scavenging capacity. The properties of these amino acids may be explained by the special capability of phenolic and indol groups to serve as hydrogen donors. In κ-CN f (23–38), four of 16 residues were tyrosine and thus may explain its high antioxidant activity (Hernandez et al. 2005).

An antioxidative peptide was isolated from milk fermented with *Lactobacillus delbrueckii* subsp. *bulgaricus* by reversed phase partition chromatography. The peptide was composed of eleven amino acid residues. The amino acid sequence of the peptide was determined as Ala-Arg-His-Pro-His-Pro-His-Leu-Ser-Phe-Met which corresponded with the amino acid arrangement from the 96th to the 106th of kappa-casein of skim milk used as raw material. Therefore, this antioxidative peptide was estimated to be a peptide formed by the lactic acid fermentation of IFO13953 (Kudoh et al. 2001). Total antioxidant activity of probiotic cheese was found to be increasing linearly 17% on 24th day and 20% on 38th day. At the end of the experiment (66th day), the total antioxidant activity of the probiotic *Lactobacillus* cells (from probiotic cheese) gained approximately the same value as the pure culture of ME-3 (26.3 vs. 26%, respectively) (Songisepp et al. 2003).

The antioxidant activities of water-soluble extracts of Cheddar cheeses prepared with *Lactobacillus* casei ssp. casei 300 and *Lactobacillus* paracasei ssp. paracasei 22 and without adjunct cultures were dependent on the ripening period. The changes in the antioxidant activity were related to the rate of formation of soluble peptides (proteolysis) in all the samples of cheeses up to fourth month of ripening (Gupta and Mann, 2009). The antioxidant peptides (< 3kDa) were fractionated and purified on C18 RP-HPLC column chromatography. The antioxidant activity of each RP-HPLC fraction was determined by ABTS (2,2’-azinobis-3-ethyl-benzothiazoline-6-sulphonic acid) radical assay method and expressed in terms of Trolox equivalent antioxidant capacity (TEAC) values (i.e. μmol of Trolox equivalence/ mg of the protein). The fraction exhibiting the highest antioxidant activity was rechromatographed on RP-HPLC to obtain a single peak, which was subjected to LC-
MS/MS. Two milk protein derived peptides have been identified: VKEAMAPK (m/z 872.5048) matching f (98-105) of β-casein and HIQKEDVPSER (m/z 1336.7034) is corresponding to αs1-casein f (80-90). Synthetic peptides were prepared using these sequences. The DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activity of synthetic peptide VKEAMAPK was comparable to commercial chemical antioxidants (BHA, t-BHQ, Ferulic acid), while it was slightly lower for synthetic peptide HIQKEDVPSER. (Gupta and Mann, 2010). Prichard et al (2010) reported that the commercial cheddar cheese peptides showed radical scavenging activity. Other research has demonstrated that cheddar cheeses enriched with herb or fruit have antioxidant activity (Apostolidis et al 2007).

**Mineral sequestering peptides**

Caseinophosphopeptides (CPPs) are bioactive peptides derived from tryptic digestion of casein and have been used in confectionary products such as breakfast foods, breads, pastry, bean curds, chocolate, caramel, juices, boiled fish, tea and mayonnaise. The CPPs have divalent metal ion sequestering activity and with binding will solubilize different ions such as Ca, Mg and Fe, along with trace elements that include Zn, Ba, Cr, Ni, Co and Se. Calcium is a mineral of special concern because it has many important functions in the human body including bone development and calcification and the prevention of hypertension and colon cancer. Calcium absorption occurs in the human body by both active vitamin D-dependent transport in the duodenum and jejunum and passive vitamin D-independent transport in the distal ileum. The affinity of CPPs to inhibit amorphous calcium phosphate precipitation by linking the seryl phosphate groups to calcium phosphate in a nanometer-sized particle results in the stabilization of the amorphous di-calcium phosphate.

CPPs isolated from Grana Padano cheese is primarily derived from three parent peptides viz. αs1-casein f (61-79)4P and β-caseins f (7-28)4P, αs2-caseins f(7-21)4P. These peptides are resistant to further hydrolysis and contain at least three closely related phosphoserine residues. The anticariogenic activity of these peptides is also observed (Ferranti et al. 1997). CPPs have been found as natural constituents in Comte’ and Cheddar cheese (Roudot et al. 1994; Singh et al. 1997). The different peptides isolated from comte cheese correspond to the fragments of the sequence β-casein f (13-28) and αs2-casein f (5-21). The cleavage of these caseins was due to plasmin, probably followed by the action of another endopeptidase and of amino-peptidases as well as lysyl carboxypeptidases (Roudot et al. 1994). Several phosphopeptides were isolated from the water soluble fraction of the diafiltration retentate. Most of the peptides isolated from the water soluble fraction of diafiltrate retentate are of β-casein origin, especially from a short region in the N-terminal half of the molecule (Singh et al. 1997).
Ardo et al. (2010) identified the large hydrophobic phosphopeptides that accumulate in semi-hard cheese, Herrgard. Eight large phosphopeptides released by plasmin hydrolysis of b casein were identified in the semi-hard cheese ie fractions f29-105, f29-107, f1-105 A1 and A2. Adt et al. (2011) most of the CPPs generated throughout in vitro digestion were monophosphorylated. 17 out of 23 polyphosphorylated CPPs identified from digested Beaufort cheese still contained the characteristic cluster sequence Ser (P)-Ser (P)-Ser (P)-glu-glu with its mineral binding sites.

**Novel cheese with bioactive properties**

Probiotic cheese is being produced on an industrial scale for the first time. ‘Festivo’ is manufactured commercially in Finland and has attracted growing interest among health-aware consumers. *Lactobacillus acidophilus* and *Bifidobacterium sp.* can be used in the manufacture of ripened semi-hard cheese. The new type of cheese, ‘Festivo’, showed good results in organoleptic analysis. ACE-inhibitory activity increased during ‘Festivo’ cheese ripening, and decreased when proteolysis exceeded a certain level during the storage period. These findings are consistent with the results of Meisel et al. (1997), who found ACE inhibition to be low in samples having a low degree of proteolysis and to increase as proteolysis proceeds up to a certain level. These results would suggest that ACE-inhibitory peptides and probably other biologically active peptides as well, are naturally formed in cheese, and remain active for a limited period before splitting into other peptides and amino acids as ripening proceeds. These peptides corresponded to the αs1-casein N-terminal peptides, f (1–9), f (1–7) and f (1–6). It is suggested that the new cheese may possess multifactorial health effects, since it contains several beneficial components such as probiotics, bioactive peptides, conjugated linoleic acid, and calcium (Ryhanen et al. 2001).

Songisepp and his co-workers (2004) developed Estonian open-texture, smear ripened, semisoft cheese “Pikantne.” This cheese was produced using probiotic *Lactobacillus fermentum* strain ME-3 with high antimicrobial activity and antioxidative properties. The probiotic strain was found to withstand the technological processing of cheese, surviving and sustaining moderate antimicrobial and high antioxidative activity throughout ripening and storage (the ripened cheese contained approximately $5 \times 10^7$ cfu/g viable ME-3 cells). Semisoft cheese “Pikantne” probiotic cheese serves as a suitable carrier of antimicrobial and antioxidative *L. fermentum* ME-3. Probiotic organisms (*Lactobacillus casei*) was added successfully in Cheddar cheeses by Ong et al. (2007) in order to provide health benefits while simultaneously producing bioactive peptides for additional health attributes. This may suggest that the proteolytic enzymes of the probiotic organisms could possibly play a role in increasing the production of ACE-inhibitory peptides in Cheddar cheeses.
Isolation and characterization of bioactive peptides from cheeses

The isolation and purification techniques are very important in bioactive peptide research. The importance of glutathione (Glu-Cys-Gly) has been noticed as early as 1888, but the bioactivities of peptides became apparent only after 1950s with the development of a new purification technology (Sewald and Jakubke 2002). Most of the protein isolation and purification techniques can be applied for bioactive peptide separation. However, because of relatively small size and molecular weight, special consideration should be given to bioactive peptides purification. Salting out and solvent extraction are often used before further purification steps. Chromatography is the most powerful technique to isolate and purify bioactive peptides. Based on different properties of peptides, different chromatographic techniques have been developed. Among them, HPLC is the most commonly used separation method. Commercially available reversed-phase columns allow for rapid separation and detection of the peptides from a mixture, whereas normal phase liquid chromatography is used preferentially for the separation of hydrophilic peptides. Ion-exchange chromatography (IEC), capillary electrophoresis (CE), and capillary isoelectric focusing (CIEF) separate peptides based on their charge properties. Size exclusion chromatography (SEC), which is also named gel filtration chromatography (GFC) in aqueous separation systems and gel-permeation chromatography in nonaqueous separation systems, is a separation method solely based on molecular size. Ultrafiltration (UF), crystallization, counter-current distribution, partition chromatography, and low-pressure hydrophobic interaction chromatography (HIC) have also been used for protein fractionation and purification (Sewald and Jakubke 2002).

Conclusion

The biologically active peptides are natural ingredients of all types of ripened cheeses. Current research has shown that microbial technology is more advantageous than any other technology for production of bioactive peptides. The identification and/or modification of lactic acid bacteria with improved properties for this purpose and their utilization in production of various types of ripened novel cheese is an obvious further objective, because the bioactive peptides containing foods have a potential market. Further there is need for increased clinical trials to be prepared with such cheeses in order to demonstrate health benefits associated with their consumption.

References:

Introduction

Lactic acid bacteria (LAB) are Gram-positive, non-spore forming, catalase-negative bacteria that are devoid of cytochromes and are of nonaerobic habit but are aero-tolerant, fastidious, acid tolerant and strictly fermentative; lactic acid is the major end-product of sugar fermentation (Axelsson et al., 1993). They are the most widely used bacteria as starter cultures for the industrial processing of fermented dairy, meat, vegetable and cereal products. Despite the starter culture addition, non-starter lactic acid bacteria (NSLAB), originating from the raw material and environment, grow out during fermentation and may reach higher numbers than the starters. Reduction of pH and conversion of sugars to organic acids are the primary preserving actions that these bacteria provide to fermented food. However, many kinds of foods are still fermented naturally, without the use of starter cultures, by autochthonous lactic acid bacteria, which form the characteristic properties of the products. These natural isolates of lactic acid bacteria from spontaneous fermentations could be used as specific starter cultures or as adjunct strains, after phenotypic and genotypic characterization and they represent a possible source of potentially new antimicrobial metabolites (Maric et al., 1984; Wouters et al., 2002; Topisirovic et al., 2006). In addition, the application of lactic acid bacteria and their antimicrobial metabolites in the prevention of food spoilage and the extension of the shelf life of food that is ready to eat, fresh-tasting, nutrient and vitamin rich minimally processed and biopreserved are the major challenges for the current food industry (Gálvez et al., 2007). The use of bacteriocin-producing lactic acid bacteria as protective strains or bacteriocins in the form of purified or concentrated compounds as biopreservatives to control undesirable bacteria remains a primary focus of researches related to food safety and quality (Havelaar et al., 2009). In the concept of functional food, especially in dairy industry, there is an increasing interest for probiotic products that contain lactic acid bacteria of intestinal origin. Probiotic lactic acid bacterial strains must be chosen according to accurate selection criteria in order to survive the transition through gastrointestinal tract and preferably colonize the intestinal tract for a sufficiently long period to achieve the desired healthy effect (Suskovic et al., 2001). One of the most important properties of probiotics is protection against pathogens in the intestinal tract of the host. The role of antimicrobial compounds produced by probiotic strains as prophylactic agents against enteric infections is crucial and well documented (Kos et al., 2008;
Organic acids

The most important and best characterised antimicrobials produced by LAB are lactic and acetic acid. The amount and type of acids produced during fermentation influence the subsequent microbial activity in the fermented material. Acetic acid, for example, is more antagonistic against yeasts compared to lactic acid. Some oxidative yeasts are able to utilize organic acids as a carbon and energy source and consequently cause spoilage through deacidification in fermented, especially plant material where they are naturally present (Daechel et al., 1987). The inhibitory effect of organic acids is mainly caused by undissociated form of the molecule, which diffuses across the cell membrane towards the more alkaline cytosol and interferes with essential metabolic functions. The toxic effects of lactic and acetic acid include

Antimicrobials from lactic acid bacteria

Antimicrobial substances produced by lactic acid bacteria can be divided into two main groups: low molecular mass substances with molecular mass <1000 Da and high molecular mass substances with molecular mass >1000 Da, such as bacteriocins. All non-bacteriocin antimicrobial substances from LAB are of low molecular mass (Collins et al., 2009).

Low molecular mass antimicrobials

The metabolites of LAB with antimicrobial activity are accumulated in their environment at the levels and proportions that depend on the species of LAB and chemical composition of the growth media. Fermentation of hexoses by lactic acid bacteria is characterized by homofermentative production of lactic acid or by heterofermentative production of equimolar amounts of lactate, acetate/ethanol and carbon dioxide. Pentoses are fermented by many heterofermentative and homofermentative LAB in the same way since phosphoketolase of homofermentative LAB is generally inducible by pentoses. Fermentation of pentoses yields equimolar amounts of lactic and acetic acid.

Most of heterofermentative species have flavoprotein oxidases, which catalyse the reduction of oxygen, resulting in the accumulation of hydrogen peroxide. During heterofermentations, products such as formic acid, acetoin, acetaldehyde and diacetyl, which possess antimicrobial activity, can be accumulated. Malic, lactic and citric acid can be further metabolised to other antimicrobial products such as acetic acid, formic acid and CO2 (Lindgren et al., 1990). The main low molecular mass metabolites of LAB and their antimicrobial spectra are shown in Table 1.

a. Organic acids

The most important and best characterised antimicrobials produced by LAB are lactic and acetic acid. The amount and type of acids produced during fermentation influence the subsequent microbial activity in the fermented material. Acetic acid, for example, is more antagonistic against yeasts compared to lactic acid. Some oxidative yeasts are able to utilize organic acids as a carbon and energy source and consequently cause spoilage through deacidification in fermented, especially plant material where they are naturally present (Daechel et al., 1987). The inhibitory effect of organic acids is mainly caused by undissociated form of the molecule, which diffuses across the cell membrane towards the more alkaline cytosol and interferes with essential metabolic functions. The toxic effects of lactic and acetic acid include
the reduction of intracellular pH and dissipation of the membrane potential (Kashket, 1987; Lorca et al., 2009).

b. Hydrogen peroxide

Antimicrobial activity of hydrogen peroxide is attributed to its strong oxidizing effect on the bacterial cell and to the destruction of basic molecular structures of cell proteins (Lindgren et al., 1990). In raw milk, hydrogen peroxide produced by lactic acid bacteria can, after being catalysed by lactoperoxidase, oxidise endogenous thiocyanate. The oxidized intermediary products are toxic to different bacteria (Daechel et al., 1987). Hydrogen peroxide production has been considered as the main metabolite of LAB that could protect against urogenital infections, especially in the case of bacterial vaginosis (Reid, 2008).

c. Diacetyl, acetaldehyde and acetoin

Heterofermentative LAB produce active acetaldehyde by decarboxylation of pyruvate. This product then condenses with pyruvate, forming a-acetolactate and it is converted by a-acetolactate synthases to diacetyl. The product of decarboxylation of a-acetolactate and reduction of diacetyl is acetoin (Collins et al., 2009; Jyoti et al., 2003). Diacetyl (2,3-butanedione) is best known for the buttery aroma that it imparts to fermented dairy products, but this property as well as high concentration needed to provide preservation of food limit the use of diacetyl as food preservative. Similarly, an acetaldehyde, usually present in fermented dairy products in concentrations smaller than necessary for inhibition of undesired microorganisms, also plays a role in controlling the growth of contaminants, together with other antimicrobial metabolites of lactic acid bacteria (Vanderbergh, 1993).

d. Carbon dioxide

The influence of carbon dioxide on product preservation is twofold. Namely, except for its own antimicrobial activity, it creates an anaerobic environment by replacing the existent molecular oxygen. The antifungal activity of CO₂ is due to the inhibition of enzymatic decarboxylations and to its accumulation in the membrane lipid bilayer resulting in dysfunction in permeability (Lindgren et al., 1990).

e. Reuterin and reutericyclin

Selected isolates of *Lactobacillus reuteri* produce two compounds, reuterin and reutericyclin, both active towards Gram-positive bacteria.

(i) Reuterin

Reuterin is a pH neutral, water soluble, low molecular weight substance, which is non-bacteriocin and resistant to nuclease, protease and lipolytic enzymes. It is active over a wide range of pH values and capable of inhibiting growth of a wide spectrum of microorganisms, but it is labile to heat (100°C for 10 minutes) (Talarico et al., 1988; Axelsson et al., 1989; Dorogosz and Lindgren, 1995; El-Ziney et al., 1999).
Bacteriocins of lactic acid bacteria

Some of the LAB produce bacteriocins, antibacterial proteinaceous substances with bactericidal activity against related species (narrow spectrum) or across genera (broad spectrum of activity) (Rogelj et al., 1994; Cotter et al., 2005). Bacteriocin biosynthesis is a desirable characteristic for strain selection as it serves as an important mechanism of pathogen exclusion in fermented foods as well as in the gastrointestinal environment. Bacteriocins are ribosomally synthesized peptides or

Reuterin was the first low molecular weight antimicrobial substance from Lactobacillus species ever to be chemically identified (Talarico and Dobrogosz, 1989), and has been intensively studied and understood (Vollenweider et al., 2003). Reuterin has been shown to exist in solution as an equilibrium mixture of three chemical compounds derived from glycerol dissimilation, namely the 3-HPA (3-hydroxy propionaldehyde) system, containing monomeric, hydrated monomeric and cyclic dimeric forms of 3-HPA (Talarico and Dobrogosz, 1989). Talarico and Dobrogosz, (1989) were the first to chemically characterize reuterin. The unique and most attractive feature of reuterin is its strong antimicrobial activity. It has been found that concentrations of reuterin in the range of 15-30 µg/ml effectively inhibit growth of Gram-positive and Gram-negative bacteria, and lower eukaryotic organisms including yeast, fungi and protozoa; while much higher concentrations are required to kill lactic acid bacteria, including L. reuteri itself (Axelsson et al., 1989; Chung et al., 1989; Casas and Dobrogosz, 2000).

(ii) Reutericyclin

Another low molecular weight compound isolated from L. reuteri LTH2584 is reutericyclin. It is a naturally occurring, amphiphilic, tetramic acid, and its optimal formation was observed between pH 4 and 5 (Holtzel et al., 2000). It has been reported to have bactericidal and bacteriostatic activity against many Gram-positive species, but does not affect Gram-negative bacteria (Ganzle et al., 2000). The mode of action of reutericyclin is to act as a proton ionophore, translocating proton across the cell membrane and dissipating the transmembrane pH potential (Ganzle and Vogel, 2003). However, reutericyclin has not been identified in reuterin producing L. reuteri strains (Ganzle, 2004).

f. Other low molecular mass antimicrobials

Other low molecular mass compounds with antimicrobial activity against Gram-positive and Gram-negative bacteria, moulds and yeasts have been described, including antifungal cyclic dipeptides, phenyllactic acid, 4 hydroxyphenyllactic acid and 3-hydroxy fatty acids (Ström et al., 2002; Sjögren et al., 2003; Valerio et al., 2004). Niku-Paavola et al. (Niku-Paavola et al., 1999) discovered new types of antimicrobial compounds produced by Lactobacillus plantarum (benzoic acid, methylhydantoin and mevalonolactone) active against fungi and some Gram-negative bacteria.

Bacteriocins of lactic acid bacteria

Some of the LAB produce bacteriocins, antibacterial proteinaceous substances with bactericidal activity against related species (narrow spectrum) or across genera (broad spectrum of activity) (Rogelj et al., 1994; Cotter et al., 2005). Bacteriocin biosynthesis is a desirable characteristic for strain selection as it serves as an important mechanism of pathogen exclusion in fermented foods as well as in the gastrointestinal environment. Bacteriocins are ribosomally synthesized peptides or
proteins with antimicrobial activity produced by many Gram-positive and Gram-negative bacteria; however, those produced by food grade LAB have received considerable attention due to their potential application in food industry as natural preservatives (biopreservatives). LAB bacteriocins are small antimicrobial peptides or proteins that possess activity towards closely related Gram-positive bacteria, whereas producer cells are immune to their own bacteriocins (Klaenhammer, 1988; Chen et al., 2003; De Vuyst et al., 2007). There are several proposed bacteriocin classifications that divide them into 3 or 4 classes: (i) lantibiotics or small, heat-stable, lanthionine-containing, single- and two-peptide bacteriocins (class I), whose biologically inactive prepeptides are subjected to extensive post-translational modification; (ii) small, heat-stable, non-lanthionine-containing bacteriocins (class II), including pediocins like or Listeria-active bacteriocins (class IIa), two-peptide bacteriocins (class IIb) and circular bacteriocins (class IIc); and (iii) bacteriolysins or large, heat-labile, lytic proteins, often murein hydrolases (class III) (Klaenhammer, 1988; Cotter et al., 2005; De Vuyst et al., 2007). Some authors (Klaenhammer, 1993; Nes et al., 1996) also proposed (iv) class IV bacteriocins that require non-proteinaceous moieties (lipid, carbohydrate) for their activity.

Lantibiotics are small (<5 kDa) peptides containing unusual amino acids lanthionine, dehydroalanine, a dehydroalanine and dehydrobutirine. According to their chemical structures and mode of action, they are subdivided into type A and type B lantibiotics (Moll et al., 1999; Guder et al., 2000; Chen et al., 2003). Class II encompasses the more common non-lanthionine-containing bacteriocins, which are non-modified, small (<10 kDa), heat stable peptides. Representatives belonging to this heterogeneous group of bacteriocins are divided into 3 subgroups. Class IIa includes pediocin-like peptides having an N-terminal consensus sequence –Tyr-Gly-Asn-Gly-Val-Xaa-Cys. Pediocin-like peptides have attracted much attention due to their specific activity against food pathogen Listeria monocytogenes (Ennahar et al., 2000). Class IIb contains bacteriocins requiring two different peptides for their activity, and class IIc contains the remaining peptides of the class, including sec-dependent bacteriocins (Chen et al., 2003). Class III bacteriocins (bacteriolysins) are large (>30 kDa), heat-labile antimicrobial proteins not as well characterised, whose mechanism of action is distinct in function as they lyse the sensitive cells by catalyzing cell-wall hydrolysis (Cotter et al., 2005). Only four LAB bacteriolysins have been genetically characterised so far (Joerger and Klaenhammer, 1990; Hickey et al., 2003), although the non-LAB bacteriolysins have been identified Class IV bacteriocins are complex bacteriocins that require non-proteinaceous moieties like carbohydrate or lipid for their activity (Klaenhammer, 1993). However, bacteriocins in this class have not been characterised convincingly, hence definition of this class requires additional characterisation (Klaenhammer, 1988; De Vuyst and Vandamme, 1994; Chen et al., 2003; Cotter et al., 2005).
Mode of bacteriocin action

Bacteriocins that are produced by LAB can be of broad or narrow spectrum, but in general, the activity is directed against low G+C Gram-positive species (Cotter et al., 2005). The antibacterial spectrum includes spoilage organisms and foodborne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus*. Wide ranges of mode of action have been described for bacteriocins, such as enzyme activity modulation, inhibition of outgrowth of spores and formation of pores in cell membrane. Most bacteriocins interact with anionic lipids that are abundantly present in the membranes, and consequently initiate the formation of pores in the membranes of susceptible cells (Moll et al., 1999; Guder et al., 2000; Chen et al., 2003; De Vuyst et al., 2007). Most of class II bacteriocins dissipate the proton motive force (PMF) of the target cell via pore formation (Venema et al., 1995). The subclass IIa bacteriocin activity depends on a mannose permease of the phosphotransferase system (PTS) as a specific target. The subclass IIb bacteriocins (two-component) also induce dissipation of the PMF by forming cation- or anion-specific pores; specific targets have not yet been identified. Finally, subclass IIc comprises miscellaneous peptides with various modes of action such as membrane permeabilisation, specific inhibition of septum formation and pheromone activity (Cotter et al., 2005).

Resistance and immunity to bacteriocins

Bacteriocin producer has developed protection mechanisms against its own bacteriocin. Two distinct systems of bacteriocin immunity in the producing cell have been identified. Protection can be mediated by dedicated immunity protein and/or a specialised ABC-transporter system involving two or three subunits that probably pump the bacteriocin through the producer membrane. These two immunity systems can work synergistically to protect the producing cells from their own bacteriocin (Klein and Entian, 1994). In the case of lantibiotic immunity, e.g. protein LanI, which is most likely localised at the cytoplasmic membrane, probably confers immunity to the producer cell by preventing pore formation by the bacteriocin. Regulation of bacteriocin production and immunity is most frequently mediated through two-component signal-transduction systems, often as part of the quorum-sensing mechanism (Quadri, 2002).

Bacteriocin-producing starter and non-starter lactic acid bacteria in food industry

Besides the well-known biopreservative effects of antimicrobial metabolites of lactic acid bacteria such as lactic acid, acetic acid, hydrogen peroxide and diacetyl, bacteriocins have the most immediate potential in food application as biopreservatives and they can be readily introduced into food without any concentration or purification (Cotter et al., 2005). Since lactic acid bacteria are
generally regarded as safe (GRAS) according to the FDA, they could be used in food production and food biopreservation.

**Bacteriocin-producing starter cultures**

The main antimicrobial effect of starter LAB, responsible for biopreservation, is the rate of acidification, but in slightly acidified products or to eliminate undesirable microorganisms that display acid tolerance, such as *Listeria monocytogenes*, the bacteriocinogenic activity could play a crucial role. The use of bacteriocin-producing starter cultures may not only contribute to food safety, but also prevent the growth of undesirable autochthonous lactic acid bacteria that produce off-flavour. This property may improve the competitiveness of the starter cultures and lead to a more controlled and standardized fermentation process as it has been shown in sourdough, fermented sausage, fermented vegetables and olives, and cheese production (Ross *et al.*, 2000; De Vuyst *et al.*, 2004; Beganovic *et al.*, 2005; Leroy *et al.*, 2006).

**Bacteriocin-producing adjunct cultures**

Bacteriocin producers can be delivered to a food product as an adjunct culture, together with the starter culture. In this case, the ability of starter adjunct to grow and produce bacteriocin in the product is crucial for its successful use. The bacteriocin-producing adjunct cultures are mostly isolated from raw milk, vegetables, cereals and other natural sources of lactic acid bacteria that are believed to contain strains essential not only for the characteristic flavour of traditional fermented products, but also with promising and useful properties such as bacteriocinogenic activity, which will make them applicable as starters. For example, *Lactococcus lactis* strain, which produces both nisin and lacticin 481, isolated from raw ewe’s milk, might be used as adjunct culture to the commercial starter in the manufacture of dairy products to inhibit or destroy undesired microorganisms (Bravo *et al.*, 2009). Adjunct culture does not need to contribute to the flavour but it is important that the starter culture is resistant to bacteriocin produced by the adjunct culture. One of the exceptions is the controlled lysis of starter culture during cheese manufacture caused by bacteriocin-producing strain, with the aim to release intracellular enzymes, needed for accelerated ripening and improvement of product flavour (O’Sullivan *et al.*, 2003; Cotter *et al.*, 2005).

**Bacteriocin-producing protective cultures**

Bacteriocinogenic protective cultures alone can be used to inhibit spoilage and pathogenic bacteria during the shelf life of non-fermented foods by producing bacteriocin *in situ* or previously cultured in growth medium and after that applied as an ingredient in food processing. Two preparations are already present on the market: ALTA™ 2341, containing pediocin PA1 produced by *Pediococcus acidilactici*, and Microgard™, a commercially available fermented milk product containing antimicrobial metabolites. In the literature different milk-based preparations such
as lacticin 3147 are described (Guinane et al., 2005). The addition of purified or semi-purified bacteriocins as food preservatives requires approval from legislative point of view. There is also a problem of costly production because of low production rates, instability and expensive downstream processing of bacteriocins. If immobilized or microencapsulated bacteriocin or bacteriocinogenic strain is applied on the food surface, much lower concentration is needed compared to the application in the whole food volume (Champagne and Fustier, 2007; Gálvez et al., 2007). Other advantages of immobilized bacteriocins are the possibility of gradient-dependent, continuous supply of bacteriocin and the protection against food components and enzymatic inactivation. The use of antimicrobial films containing immobilized bacteriocins for the development of antimicrobial packaging is a recently developed technique (La Storia et al., 2008; Papagianni and Anastasiadou, 2009).

**Conclusions**

The ability of lactic acid bacteria to produce antimicrobial substances has historically long been used to preserve foods. Due to their fermentative metabolism, LAB produce organic acids, hydrogen peroxide, carbon dioxide and diacetyl. A few strains produce specific antimicrobial substances like reuterin, pyroglutamic acid, cyclic dipeptides and phenyl lactic acid etc. Except for these general antimicrobial substances, many strains have been found to produce bacteriocins. These have often a more defined antimicrobial spectrum ranging from only related strains to a wide variety of Gram-positive and Gram-negative bacteria. Bacteriocinogenic protective cultures alone can be used to inhibit spoilage and pathogenic bacteria during the shelf-life of non-fermented foods by producing bacteriocins *in-situ*. Many of these antimicrobials are thought to have potential applications as preservatives.

**Table 1.** Low molecular mass antimicrobial metabolites of lactic acid bacteria (Modified from Daechel et al., 1989; Lindgren et al., 1990; Vanderbergh et al., 1993)

<table>
<thead>
<tr>
<th><strong>Compound</strong></th>
<th><strong>Microorganisms producers</strong></th>
<th><strong>Antimicrobial spectrum</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>lactic acid</td>
<td>all lactic acid bacteria</td>
<td>yeasts Gram-positive bacteria Gram-negative bacteria</td>
</tr>
<tr>
<td>acetic acid</td>
<td>heterofermentative lactic acid bacteria</td>
<td>yeasts Gram-positive bacteria Gram-negative bacteria</td>
</tr>
<tr>
<td>diacetyl</td>
<td>variety of genera of lactic acid bacteria including: <em>Lactococcus, Leuconostoc, Lactobacillus</em> and <em>Pediococcus</em></td>
<td>yeasts Gram-positive bacteria Gram-negative bacteria</td>
</tr>
<tr>
<td>acetaldehyde</td>
<td></td>
<td></td>
</tr>
<tr>
<td>acetoin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hydrogen peroxide</td>
<td>all lactic acid bacteria</td>
<td>yeasts Gram-positive bacteria</td>
</tr>
<tr>
<td>Substance</td>
<td>Bacteria</td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>carbon dioxide</td>
<td>heterofermentative lactic acid bacteria</td>
<td></td>
</tr>
<tr>
<td>reuterin</td>
<td>Lactobacillus reuteri</td>
<td></td>
</tr>
<tr>
<td>reutericyclin</td>
<td>Lactobacillus reuteri</td>
<td></td>
</tr>
<tr>
<td>cyclic dipeptides</td>
<td>Lactobacillus plantarum Lactobacillus pentosus</td>
<td></td>
</tr>
<tr>
<td>3-phenyllactic acid, 4-hydroxyphenyllactic acid</td>
<td>Lactobacillus plantarum, Lactobacillus alimentarius, Lactobacillus rhamnosus, Lactobacillus sanfranciscensis, Lactobacillus hilgardii, Leuconostoc citreum, Lactobacillus brevis, Lactobacillus acidophilus, Leuconostoc mesenteroides</td>
<td></td>
</tr>
<tr>
<td>3-hydroxy fatty acids</td>
<td>Lactobacillus plantarum</td>
<td></td>
</tr>
<tr>
<td>benzoic acid, methylhydantoine, mevalonolactone</td>
<td>Lactobacillus plantarum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gram-negative bacteria</td>
<td></td>
</tr>
<tr>
<td></td>
<td>fungi</td>
<td></td>
</tr>
<tr>
<td></td>
<td>fungi</td>
<td></td>
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<tr>
<td></td>
<td>fungi</td>
<td></td>
</tr>
</tbody>
</table>

**References**


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Introduction

The quality of raw milk used for cheese making is perhaps the most important consideration when quality cheese is desired. Cheesemakers should pay great attention to raw milk quality as this will in turn dictate cheese quality. The first step in cheese manufacturing is analysis and quality control of milk, since these factors greatly influence the economics of cheese manufacturing, composition of cheeses and their sensory qualities. The amount of milkfat has traditionally served as the basis of payment of milk but now it is determined by levels of fat, protein and quality factors. The quality of finished product depends on the quality of the raw material. Therefore, it is recommended that the quality of milk for cheese making should be of highest or best quality in order to get a cheese of desirable quality.

The different factors that should be taken into consideration when milk is to be taken for cheese making are:

- The amount and composition of milk constituents
- Abnormal milk
- Changes in milk after production
- Inhibitory substances and other residues
- Micro flora of milk

The amount and composition of milk constituents

The composition of milk is closely linked to yield and properties of resultant cheese as it influences coagulum properties and its structure which ultimately influences yield, flavor and texture of cheese.

Milk proteins

Casein is the principal milk protein of interest to the cheesemaker. Variations in concentration of total casein and relative proportion of individual casein, genetic variants of the casein present, size of the casein micelle and mineral make up of casein micelles have profound effect on the cheese making properties of milk as:

- Rennet clotting time
- Curd strength
- Syneresis of curd
- Yield of cheese
- Non specific proteolysis of cheese
- Composition of the resultant cheese
- Organoleptic quality of resultant cheese
For manufacturing of cheese, milk that is high in casein and low in serum protein and lactose is desirable, since during cheese making major portion of whey proteins and lactose is lost in whey. Higher concentration of casein in milk has been found to give curd of higher strength, rapid rate of curd firming, longer syneresis time, higher cheese yield with the cheese having a firm body and texture. Higher whey protein in milk delays Rennet Coagulation Time and produces a weak coagulum. The different genetic variants of α, β and K caseins have been found to influence the technological suitability of milk for cheese making. Cheese milk containing B variant of β- and K-casein gave shorter RCT, higher curd strength, improved syneresis, increased cheese yield and cheese with better organoleptic quality than milk devoid of this variant.

Table 1. Effect of genetic variants of casein on cheese making characteristics

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Characteristics affected</th>
<th>Casein variants involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acid production</td>
<td>Slow acid production with αs1-Cn A variant than B and C</td>
</tr>
<tr>
<td>2.</td>
<td>Rennet action</td>
<td>1. αs1-Cn B + β-Cn A + K-Cn B</td>
</tr>
<tr>
<td></td>
<td>Slower</td>
<td>2. αs1-Cn B + β-Cn AC + K-Cn AB</td>
</tr>
<tr>
<td></td>
<td>Faster</td>
<td>1. αs1-Cn B + β-Cn B + K-Cn AB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. αs1-Cn B + β-Cn B + K-Cn B</td>
</tr>
<tr>
<td>3.</td>
<td>Curd firmness</td>
<td>1. αs1-Cn BC &gt; αs1-Cn C &gt; αs1-Cn B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Other variants of β-Cn &gt; β-Cn A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. K-Cn B &gt; K-Cn AB &gt; K-Cn A</td>
</tr>
<tr>
<td>4.</td>
<td>Degree of proteolysis</td>
<td>Least proteolysis with β-Cn B</td>
</tr>
<tr>
<td>5.</td>
<td>Cheese yield</td>
<td>Higher yield with K-Cn B</td>
</tr>
</tbody>
</table>

Milk fat

In the manufacture of certain varieties of cheese (e.g. Roquefort), sheep milk is preferred because such milk contains significantly higher amounts of C₆ - C₁₀ fatty acids. Part of the flavor difference between blue cheese made from cow's milk and Roquefort made from sheep's milk is clearly due to the higher concentrations of caprylic acid and capric acid in the latter, resulting from their higher proportions in ewe's milk fat. These fatty acids have been found essential for peppery flavor characteristics of this type of cheese. Variations in the quantity and quality of fat in milk have direct influence on cheesemaking as concentration of fat in milk has been reported to influence coagulum development when milk is renneted. Increase in the fat content of milk prolongs the time required for initial clot formation whereas the time required between initial clot formation and cutting is reduced. However this is unlikely to change the overall RCT. Syneresis of curd which plays an important role in the cheese making and quality of cheese is very much affected by the fat content of milk. Increase in the fat content of milk results in reduced syneresis, thus longer draining times are required to achieve desired moisture content in cheese. On the other hand cheese made from no fat or low fat milk has problem of moisture retention and it dries out fast to give a hard and dry body.
The curd obtained from milk containing low levels of fat is more leathery and the resultant cheese lacks mellow velvetiness. Too high a fat content in milk, on the other hand, yields a product which is too soft, buttery and too greasy and may show fat leakage during ripening. (It is not the absolute amount of fat but it is the C/F ratio adjusted in milk which decides the body and texture characteristics of cheese). The fat content of milk is also directly associated with the yield of cheese as fat and casein constitute >90% of the total solids of cheese. The cheese yield / kg of fat used decreased with increase in fat content of milk as high fat milk usually contains less casein in proportion of fat than does milk less rich in fat. The extent of decrease in the yield /kg fat is less marked as fat increases owing to relatively reduced fat losses in high fat milk. Milk fat acts as a reservoir for fat soluble precursors, intermediate and flavor compounds involved in flavor development in cheese whereas cheeses made with skim milk do not develop typical cheese flavor as they lack the richness and delicacy of flavor contributed by milk fat and liberated fatty acids.

The physical and chemical make up of milk fat plays a significant role in cheese making. The size of fat globules and the composition of milk fat influences incorporation of fat in curd and thus affect fat recovery in cheese. Small size globules are easy to incorporate in curd than larger ones which when in molten conditions are easily pressed out of curd. The fat is either lost in whey or occupies the open space in curd to impart a greasy mottled defect. For better incorporation in the curd milk fat with higher melting point is preferred. Composition of fat has influence on flavor genesis. Milk fat is unique as it is a source of methyl ketones and lactones which contributes to flavor of certain cheeses which would not be produced from most animal and vegetable fat.

**Milk salts**

The calcium content (colloidal, soluble and ionic) of milk greatly influences the RCT, firmness of curd and together with phosphate is important for the drainage of whey. The level of Ca++ retained in the curd also influences the body and texture characteristics of cheese. Normal milk contains adequate amount of calcium which is needed for proper coagulation of milk by rennet. Variations in concentration of calcium as well as magnesium, phosphates and citrates and sodium have a direct influence on rennet clotting of milk. High soluble phosphate, citrates and sodium and low soluble Ca++ and magnesium and low proportion of casein bound Ca++ have been found to give slow coagulation of milk by rennet. In view of importance of Ca++ in coagulation of milk, addition of 0.01-0.02% CaCl₂ to slow renneting and overheated milks to assist rennet activity is a common practice. However, addition of excessive amount of CaCl₂ to milk imparts a bitter taste to cheese. Certain trace elements such as manganese, copper, cobalt, zinc have been found to stimulate the growth of lactic acid bacteria and their presence has a favorable effect on the maturation of cheese.
Abnormal milks

Abnormal milk is the general term used to describe any type of milk that differs markedly from normal milk and usually includes mastitis milk, colostrums and late lactation milk. A cheese maker wants more or less complete absence of abnormal milk in the supplies of milk as they are unsuitable for cheesemaking on account of:

- Factors or conditions resulting in slow starters
- Slow coagulation with rennet and formation of weak curd
- Contamination with common, fault producing organisms, resulting in off-flavoured cheese
- Presence of organisms which produce more rare and late developing faults such as red spots
- Abnormal chemical composition which may affect both ripening and development of flavor

Mastitis milk

Alkaline pH, high somatic cell count and abnormal composition results in slow growth of starter activity during cheese making. Altered chemical composition of casein, lower ionic calcium and presence of plasma protein alpha macroglobulin results in delayed rennet action resulting in weak curd which is less cohesive and less firm. This results in reduced yield and higher N and fat losses in whey. Also this results in poor cheese flavor and body-texture and results in abnormal fermentation (gassiness, blowing etc.).

Colostrum

- It differs from that of normal milk with respect to its composition and physic chemical properties. Use of colostrums is unsuitable for cheese making as
- It gives curd which lacks elasticity and shrinkability
- The curd retains more moisture and its drying is difficult
- It favours growth of undesirable organisms that may produce gas holes, off flavors and taints
- Presence of high somatic cells and natural inhibitors in such type of milk may interfere with the starter activity especially when cheese is made from raw milk.
- Bulking of colostrums with normal milk upto 10% of the total volume of milk however has been reported to have no adverse effect on cheese making.

Late lactation milk

It is generally accepted that milk after 8 months of calving is undesirable for cheese making owing to its altered chemical composition and physic chemical properties. This type of milk resembles biochemically subclinical mastitis milk and therefore its effect on cheese making and cheese quality would be similar to that of cheese prepared from mastitic milk.
Changes in milk quality after production and before start of cheese manufacturing

Milk is subjected to physical, chemical, microbiological and organoleptic alterations during the period that elapsed between milk production and cheese making. Thus milk after production may show:

Development of off flavor: Off flavor of any kind in milk are carried over into the cheese and because of concentration in curd where it is either associated with fat or protein, it may become pronounced in the finished cheese and thus affect the quality of cheese. Off flavors may arise because of consumption of certain weeds and feeds or absorbed by milk during or after milking or caused due to biochemical changes in milk after production.

Acid production: Raw milk on standing at ambient temperature shows increase in acidity on account of lactose fermentation by lactic acid bacteria and when the level of acidity reaches to a point >0.2%LA where milk tends to clot on boiling.

Lipolysis, proteolysis: Lipolysis in milk prior to its conversion into cheese has detrimental effects on process of cheesemaking and also on the quality of the finished cheese. As the effect of initial lipolysis in milk is carried over to the curd and thus to the cheese, it contributes to the overall lipolytic changes that takes place in the cheese. As a consequence the resultant cheese may develop rancidity defect. Lipolyzed milk has been shown to give weak curd formation and delayed coagulation with rennet. The two reasons ascribed for weak curd formation are –

- the free fatty acids forming an insoluble soap like substance with calcium thus depleting the amount of free calcium available for coagulation
- the free fatty acids may combine with the casein micelles complex and block the cleavage of chymosin sensitive bond required for coagulation of milk.

In addition to the above effects, lipolysis in milk may also affect starter activity. The two possible mechanisms implicated in reduced starter activity in lipolyzed milk are

- reduction in surface tension caused by liberated fatty acids
- certain FFAs such as C8-C12 have toxic effect on certain strains of starter

Nowadays, milk is often stored under refrigeration for 2-3 days before conversion of raw milk into cheese. This increases the possibility of growth of psychrotrophs and also brings about some changes in the composition of milk. These changes in milk results in

- increased rennet coagulation time
- slow drainage
- increased curd losses in whey and decrease in cheese yield
- increased processing time
- cheese sometimes show poor grading on account of defective body and texture
Changes in casein micelles and salt balance: In today’s practice, cooled raw milk is held in bulk tanks for extended periods which not only encourages psychrophilic bacteria but also modifies the casein fractions like β-Cn, αs1-Cn and K-Cn and also alters the calcium as well as phosphorous level. These changes in turn results in:

- increased rennet coagulation time
- decreased curd strength or firmness
- increased curd losses in whey and decrease in cheese yield

Effect of inhibitory substances in milk on cheese making

Natural inhibitory substances in milk: The natural inhibitory systems in raw milk include immunoglobulins, lactoferrin, lysozyme and lactoperoxidase-thiocyanate-hydrogen peroxide system. Presence of such inhibitory substances can influence cheesemaking particularly when cheese is made from raw milk as these systems have been found active against lactic streptococci affecting their multiplication and acid production. Lactic cultures can interact with immunoglobulins to form aggregates or clumps. As the cell produces acids, casein coagulates around these clumps and they settle out of the milk forming a sludge. Acid production is inhibited because diffusion of acid out of sludge is limited, causing acid inhibition of the culture before milk is properly acidified. Lactoferrin is an iron binding protein that inhibits bacteria by chelating iron and making it unavailable for bacterial growth. But inhibition of starter culture by lactoferrin is not significant as it competes with citrate for binding the iron. Lysozyme inactivates bacteria by cleaving the glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan layer of cell wall. Bovine milk contains a very low level of lysozyme that is unlikely to inhibit starter culture growth strains of lactic acid bacteria can produce sufficient hydrogen peroxide under aerobic conditions and a self inhibitory effect may be observed during cheese making, if starter consists of such strains.

Antibiotic residues: Antibiotics may gain entry into milk as a result of treatment of the animal, usually for mastitis. The presence of residual antibiotics in milk can result in partial or complete inhibition of starter culture which can result in:

- slow whey drainage
- high moisture in cheese
- early and late blowing
- weak and pasty body
- various types of taints
- cracks, open texture and sponginess

The inhibitory action of the residual antibiotics on starter is dependent on factors such as:

- type of antibiotic
- its residual concentration present in milk
- the type of starter involved and the type of cheese.
The order of inhibitory effect of different antibiotics against starter, in general, is as follows:

Penicillin>Subtillin>Streptomycin>Aureomycin>Bacitracin>Chloromycin (weakest)

The cheese starters comprising of S. cremoris, S. thermophilus, Propionibacterium shermanii are most susceptible to presence of residual antibiotics than those consisting of S. lactis, S. faecalis, L. casei.

**Residues of detergents and sanitizers:** In general detergents pose few problems because they are usually rinsed from equipment, permitting very little contamination of milk to occur. Also, very high residue concentrations are requires impairing the organoleptic or technological properties of the milk. On the other hand, sanitizers pose greater problems as they are often the last solution on a surface before the milk and some of them have organoleptic and technological effects at low residual concentration. Some of the cheesemaking properties likely to be affected by the presence of detergent/sanitizer residue are:

- acid production inhibition
- reduction in curd firmness
- delayed/inhibition of rennet action
- partial/full inhibition of proteolysis
- flavor of cheese may be affected

**Residues of pesticides and mycotoxins:** From public health point of view, it is important that the milk used for cheesemaking is free from insecticides, pesticides, mycotoxins and heavy metals. Though the milking animal operates as a biologic al filter, feeding of heavily contaminated feeds and fodders with such agents can cause high levels of these substances in milk and these in cheese. The main mycotoxin of concern that might occur in milk is M1 as it is a metabolite of the highly carcinogenic aflatoxin B1 and also has structural resemblance to B1. During cheese manufacture there is partitioning of M1 between cheese curd and whey. But almost 97-98% comes in curd.

(Table 2). **Microflora of raw milk and their relation to cheese defects**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Cheese defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogens</td>
<td>Diseases</td>
</tr>
<tr>
<td>Clostridium</td>
<td>Blown cheese</td>
</tr>
<tr>
<td>Coliforms</td>
<td>Blown cheese</td>
</tr>
<tr>
<td>Pseudomonads</td>
<td>Proteolytic and lipolytic activity</td>
</tr>
<tr>
<td>Spores- Bacillus cereus and Bacillus subtilis</td>
<td>Proteolytic activity</td>
</tr>
<tr>
<td>Psychrotrophic bacteria</td>
<td>Proteolysis and lipolysis</td>
</tr>
<tr>
<td>Thermotic bacteria</td>
<td>Taints e.g. <em>S. faecalis</em></td>
</tr>
<tr>
<td>Antibiotic producing organisms</td>
<td>Starter growth problems</td>
</tr>
<tr>
<td>Yeasts</td>
<td>Blown curd</td>
</tr>
</tbody>
</table>
With a view to exercise control over quality of incoming milk as well as at different stages of handling at the cheese factory, it is necessary to have its testing both for chemical composition and microbial population.

**Table 3. Tests for quality check of milk**

<table>
<thead>
<tr>
<th>Quality tested</th>
<th>Recommended test</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavor and test</td>
<td>Organoleptic</td>
<td>Daily</td>
</tr>
<tr>
<td>Acidity/pH</td>
<td>COB, titration, pH</td>
<td>Daily</td>
</tr>
<tr>
<td>Fat</td>
<td>Gerber, milkotester, infrared analyzer</td>
<td>Daily</td>
</tr>
<tr>
<td>Casen/crude protein</td>
<td>Formal titration/infrared analyzer</td>
<td>Daily</td>
</tr>
<tr>
<td>SNF</td>
<td>Lactometer, infrared analyzer</td>
<td>Daily</td>
</tr>
<tr>
<td>Mastitis</td>
<td>Conductivity, catalase, cell count</td>
<td>Weekly/fortnightly</td>
</tr>
<tr>
<td>Bacterial count</td>
<td>MBRT or 1hr resazurin</td>
<td>Weekly/fortnightly</td>
</tr>
<tr>
<td>Potential fault producing organism count</td>
<td>Coliform count, anaerobic count, yeasts, psychrotrophs</td>
<td>Need based</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Charm test, starter activity test</td>
<td>Weekly/fortnightly</td>
</tr>
<tr>
<td>Rennetability</td>
<td>Coagulation with rennet</td>
<td>Need based</td>
</tr>
<tr>
<td>Preservatives</td>
<td>Dye reduction test, test for formalin</td>
<td>Weekly/fortnightly</td>
</tr>
</tbody>
</table>

**Conclusion**

Milk as a starting material for cheese making exerts a decisive influence on the course of manufacture and affects yield and quality of resultant cheese. The cheese making properties of milk are sensitive to variations and changes in the quality of raw milk could influence cheesemaking, entailing modifications in the existing process of manufacture or may disrupt normal manufacturing operations or may give altered product yield, composition and quality. Hence there is a need to use high quality milk for cheese making.

**References**

The interest in probiotics has been growing enormously during the last few years in view of their multiple health promoting physiological functions. They are currently being explored as biotherapeutics for human health applications to manage chronic diseases. Functional foods have emerged as a newer approach to improve human nutrition and well-being in an environment where metabolic inflammatory disorders due to sedentary lifestyle and ageing population are considered as a threat to the wellbeing of the society worldwide including Asia where dietary habits are changing rapidly. Diet-related chronic diseases such as metabolic inflammatory disorders such as obesity, cardiovascular diseases (CVD) and type II diabetes (DM-2) have dramatically increased leading to concomitant increase of healthcare and other societal costs. The advent of health-promoting functional foods has been facilitated by fast accumulating scientific knowledge about the metabolic and genomic effects of diet and specific dietary components on human health. As a result of this, opportunities have arisen to formulate food products which deliver specific health benefits, in addition to their basic nutritional value. Probiotics are now being intensively investigated as an integral component of functional foods to act as therapeutic armamentarium of inflammatory metabolic disorders as an adjunct to the traditional anti-inflammatory and immune-suppressive agents. Action of probiotics on the host immune system has entered a new and fascinating phase of research in search for anti-inflammatory agents. It is likely to offer novel and useful means to modulate host immunity for protection from or treatment of a wide variety of human diseases including metabolic disorders like obesity, DM-2 and cardiovascular diseases. The immune system is extremely complex and amazingly important for maintaining perfect health. Inflammation is one of the most important defensive methods employed by the immune system to fight against infections and tissue damage, thereby, preventing the spread of infection and pathological changes to the rest of the body.

Although, inflammation is a natural defense mechanism against toxic components such as oxidized proteins and lipids, it has become one of the hottest areas of medical research due to the fact that 'Inflammation acts as a secret killer'. It presents a major hazard to individuals afflicted by several of inflammatory diseases such as IBD, CD, RA, metabolic syndrome including CVD. It is now well recognized that inflammation plays a central role in the pathogenesis of metabolic diseases.
Evidence linking inflammation to insulin resistance derives from both epidemiological studies and experimental data in humans and animal models. It is well known that the prevalence of diabetes, obesity, and Metabolic syndrome all increase with age. Inflammation disturbs the homeostasis existing between anti and pro-inflammatory cytokines. The increased level of pro-inflammatory cytokines like IL-6 and TNF-α increases the hepatic synthesis of acute phase proteins like fibrinogen, C reactive protein etc. and at the same time, they decrease the synthesis of high density lipoprotein (HDL). Few studies have shown that the pro-inflammatory mediators, particularly TNF-α, can induce a procoagulant state by eliciting tissue factor production on the surface of vascular endothelium and monocytes, down regulating the protein C anticoagulant pathway and stimulating thrombin and fibrin formation. Therapeutic approaches that reduce the levels of pro-inflammatory biomarkers and address traditional risk factors are specifically important in preventing cardiovascular disease and, potentially metabolic disorders. It has been shown that some probiotic organisms can modulate the in vitro expression of pro and anti-inflammatory molecules in a strain-dependent manner. Many probiotic effects are mediated through immune regulation, particularly through balance control of pro-inflammatory and anti-inflammatory cytokines. These data show that probiotics can be used as innovative tools to alleviate intestinal inflammation, normalize gut mucosal dysfunction, and down-regulate hypersensitivity reactions. Probiotics exhibit adequate fitness to survive and replenish physiological microflora, suppress pathological microflora and modulate host immune system. The consumption of probiotics helps to decrease the level of pro-inflammatory biomarkers which in turn helps to reduce the fibrinogen level in the blood.

The key issue of understanding the functionality of probiotic stains is the identification of appropriate biomarkers for their health benefits both under in vitro and in vivo conditions. Quantification of genes at transcriptional levels is an important criteria to know gene functionality and abnormal alterations in regulation that may result in a disease state since cellular functions are regulated by changes in gene expressions. Genomics- based studies reveals numerous bacterial cell-surface-associated proteins with intestinal cell and mucus binding functions. Relative expression of probiotic marker genes using one of the genomic approaches like Real Time PCR, Microarray etc. forms an important parameter to select potential probiotic strain which could be finally used in human clinical trials to ascertain its probiosis before its exploitation in functional foods. Similarly, specific activity can also be analysed using proteomics and a more promising metaproteomic approach. Combination of Genomics, Metagenomics and Proteomics will enable us to unravel the role of probiotics for gut health. The strategies based on of all these approaches towards understanding the functionality of indigenous probiotic strains will be discussed in this presentation.
SENSORY EVALUATION OF CHESES

Kaushik Khamrui

Introduction

Sensory evaluation is the measurement of a food product’s quality based on information received from the five senses i.e. sight, smell, taste, touch, and hearing. 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. Until recently, Cheddar was the post widely produced and plentifully available natural cheese worldwide. The burgeoning pizza market has led to the emergence of Mozzarella cheese as the contended for the honour of most available cheese worldwide. However, the rapid rise of Mozzarella does not diminish the importance of Cheddar cheese, which continues to be strong as a stand alone product and as an important ingredient for the food industry. (Clark et al., 2009)

Desirable attributes of cheddar cheese

**Colour:** the colour of Cheddar cheese should be uniform throughout. The most desired colour of Cheddar cheese is very light straw for the natural coloured cheese or deep straw or yellow orange for the medium coloured cheese. The cheese should be translucent, that is, it should appear as if one could actually see into the cheese for a short distance.

**Finish and appearance:** Cheese with a desirable finish should show flat, parallel ends; square, even edges; an evenly-folded, neat, close fitting bandage or wrapper free from wrinkles; a clean, thin, uniform, close-adhering plastic film/parafin, showing no blisters or scales; and freedom from cracks, mold, rotten spots, or soiled areas.

**Body and texture:** the desired body and texture of cheddar cheese is that which yields a full, solid, close-knit plug possessing smoothness, meatiness, waxiness and silkiness and which is entirely free from gas holes. Such cheese slices well.

**Flavour:** High quality Cheddar cheese has a characteristic clean, delicate, pleasing aroma and a nutty flavour.

**Score card of cheddar cheese**

The weightage given to different attributes is given in score card (Table 1)
Scoring technique of cheddar cheese

Tempering cheese: Cheese should be kept in a room at 10-15°C for a sufficient of time to secure a uniform temperature throughout all parts of the cheese. A plug taken from warm cheese appears weak bodied while a plug from cold one will appear brittle or corky. Hence, to know the true characteristics of cheese, tempering is must before scoring.

Sampling: It is done with a cheese trier. The edges of a cheese trier are sharper than a butter trier. A trier that cuts a larger plug has an advantage over one of small diameter because it is much easier to detect the degree of openness and the colour defects on the larger plug. Cheese trier is inserted in the middle of the cheese block, rotated at 180° and withdrawn. After drawing a plug of cheese, break the upper 2 cms and put in the hole again from where the plug was drawn.

Sequence of observations

a) Aroma: Immediately after withdrawing the plug of cheese from the block pass it slowly under the nose and inhale strongly to ascertain the aroma. Then examine the remaining plug carefully. Make mental note of all these observations.

b) Colour: Note whether the colour is bright, clear or dull; whether it is uniform, free from mottled or light and dark portions, or it has seams or faded areas surrounding the mechanical holes.

c) Openness: Observe the nature and extent of openness in the cheese. Note whether the holes are regular, angular, rounded, large, or small. Observe also the luster or shine of their inner surfaces and note if they are dry or wet.

d) Body and texture: Hold the ends of the plug by the fore-fingers and the thumbs of the two hands and bend the plug slowly into a semi-circle, observing when it breaks and the nature of the break. Observe carefully whether the plug shows a resistance towards bending and finally breaks suddenly, or bends one half of one third and eventually tears apart slowly. Take one of the broken pieces between the thumb and the fingers and work it up into a uniform mass, observing its resistance to the pressure of the thumb and the fingers. Spread the mass thinly over the palm of the hand with the thumb and observe whether the mass feels smooth, silky, waxy and fine or whether it is sticky, pasty, mealy or crumbly. Reassemble the particles; compress them into a ball, noting meanwhile the response of the cheese to its manipulation. Also note the behaviour of cheese while biting, chewing, mastication and swallowing.

e) Flavour: Place the worked mass (ball) under the nose and observe the aroma. Compare this aroma with that noted when the sample was first removed from the block using the trier. Place a small portion of the unworked plug into the mouth, chew it up to the semi-solid state, roll into the mouth, expectorate and note the
flavour. Rinse the mouth occasionally with lukewarm saline water (1%), which cleans the mouth satisfactorily to the pervious cheese flavours.

**Defects in cheddar cheese**

Various sensorial defects in Cheddar cheese their causes and remedial measures are presented in Table 2, 3 & 4.

**Sensory attributes of mozzarella cheese**

It is a soft unripened variety of cheese of Italian origin. It is produced from whole or partly skimmed milk to which small amounts of starter or organic acids are added followed by rennet extract. The curd is cut, allowed to firm up in the warm whey with occasional stirring and then the whey is drained off. When the curd has developed the desired plasticity and fibrous texture the curd is milled. The curd pieces are immersed in hot water kneaded, stretched and moulded. Salting of cheese in brine solution is done for few days. The cheese can be consumed after the brine treatment is complete.

**Colour and appearance**

Mozzarella cheese should have a uniform white to light cream colour. Faulty manufacturing method and microbial contamination may sometimes cause colour defects in the product. Use of too high salt may cause discoloration. Development of browning may be caused by using starter culture containing only thermophilus. Contamination with pseudomonas spp. causes development of superficial reddish marks.

**Body and texture**

Mozzarella cheese should have a soft, elastic, waxy and moist body with typical structure of cooled curd cheese. It should have a fibrous texture with no gas holes. It should possess a good slicing as well as melting properties. Use of too high salt or growth of *Lactobacillus casei* may cause poor melting quality. Undesirable microbial contamination may cause development of defects, like pigmentation, hole formation and other textural defects. Rapid evaporation of moisture from the surface leads to the development of granular texture.

**Flavour**

Bland, pleasant but mildly acidic with slightly salty taste is the characteristic of mozzarella cheese. Buffalo milk cheese is a more piquant and aromatic then cow milk cheese. Microbial contamination, particularly with pseudomonas species may lead to the development of flavour defects like putrid small, bitter flavour etc. other flavour defects may be absorbed or chemical nature.
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 flat, weedy and cooked flavour are not very objectionable. The scores are thus based on the nature of defect and its intensity. Finally 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 1. Score card for sensory evaluation of cheddar cheese

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Perfect Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavour</td>
<td>45</td>
</tr>
<tr>
<td>Body and Texture</td>
<td>30</td>
</tr>
<tr>
<td>Finish (Appearance and make-up)</td>
<td>15</td>
</tr>
<tr>
<td>Colour</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Colour & appearance defects in cheddar cheese

<table>
<thead>
<tr>
<th>Defects</th>
<th>Causes</th>
<th>Remedies</th>
</tr>
</thead>
</table>
| Acid-cut: bleached, or dull looking | Excessive acid development in whey or packaging stage  
Non-uniform moisture distribution in cheese | Monitor acid development  
Ensuring consistent moisture retention in the curd |
| Mottled appearance: Irregularly shaped light & dark areas on the cheese surface | Combining curds of different colours, batches, moisture content  
Uneven acid development  
Unwanted microbial growth | Avoid adding starter after colour incorporation  
Attempt to cut the curd into uniform sized particles  
Avoid drying during matting, Cheddaring, etc. |
| Pinking: develops a pink colour on the surface | 1. Oxidation of annatto colour | Avoid storing of cheese under fluorescent lighting  
Allowing proper development of acid to develop during cheese making  
Pack cheese in good O2 barrier film |
<table>
<thead>
<tr>
<th>Seamy: shows light coloured lines around curd pieces</th>
<th>Exudation of milk fat from curd pieces due to excessive forking, too-warm temps &amp; lack of salt dissolution Over-stirred set</th>
<th>Wash ‘greasy’ curd &amp; drain thoroughly</th>
</tr>
</thead>
<tbody>
<tr>
<td>White specks: granules or small hard mineral or protein deposits</td>
<td>If young cheese, results from Ca-lactate complex formation If in aged cheese, derived from proteolysis &amp; crystallization of tyrosine</td>
<td>Use method that limit the levels of LA &amp; Ca in the serum of cheese Limit the fermentation through selection of appropriate cultures Minimize post-packing acid development</td>
</tr>
<tr>
<td>Moldy appearance</td>
<td>Growth of mold on cheese</td>
<td>Ensure airtight seals on the cheese package Avoid O2 in the packages by vacuum or CO2 or N2 gas flushing</td>
</tr>
</tbody>
</table>

**Table 3. Body & texture defects in cheddar cheese**

<table>
<thead>
<tr>
<th>Defects</th>
<th>Causes</th>
<th>Remedies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crumbly, mealy/grainy</td>
<td>Excessive acid production &amp; low moisture retention in cheese</td>
<td>1. Avoid ripening at higher temps 2. Control acid development and moisture level in curd</td>
</tr>
<tr>
<td>Curdy or rubbery</td>
<td>Inadequate curing condition</td>
<td>Optimize ripening temp &amp; time</td>
</tr>
<tr>
<td>Pasty, sticky or wet</td>
<td>1. High moisture retained in curd</td>
<td>1. Control acid development in</td>
</tr>
</tbody>
</table>

177
<table>
<thead>
<tr>
<th>Defects</th>
<th>Causes</th>
<th>Remedies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitter</td>
<td>Excessive moisture Low salt Proteolytic SC strains Microbial contaminants Excessive acidity</td>
<td>Use carefully selected culture Reduce starter amount Monitor salting levels Upgrade milk quality Improve sanitation</td>
</tr>
<tr>
<td>High acid</td>
<td>Development of excessive LA Excessive moisture Use too much starter Use high acid milk</td>
<td>Reduce ripening time Reduce starter amount Monitor salting level Cook to slightly higher temperature Avoid piling crud slabs too high or too soon</td>
</tr>
<tr>
<td>Fermented (vinegar-like)</td>
<td>Heterofermentative Lactobacilli</td>
<td>Improve cooling Short wash pasteurizer every 8-12 hrs Review milk quality</td>
</tr>
<tr>
<td>Flat (lacks flavour)</td>
<td>Lack of acid production Use milk low in fat Excessively high cooking temp Using too low curing temp Too short curing period</td>
<td>Check starter activity Increase starter amount Increase curing temp Lengthen curing period Standardize cheese milk for fat content</td>
</tr>
<tr>
<td>Fruity</td>
<td>Certain strains of S. Lactis or S. diacetylactis Low acidity Excessive moisture Low salt level Poor milk quality</td>
<td>Eliminate lactic acid strains that produce ethanol Monitor starter activity Check salting procedures Upgrade milking quality</td>
</tr>
</tbody>
</table>

Table 4. Flavour defects in cheddar cheese
<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
<th>Remedies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rancid Milk</td>
<td>Lipase activity from microbial lipases, accidental homogenization of raw milk, late fermentation or mastitic milk</td>
<td>Standardize Cheddaring procedure, avoid excessive agitation, improve sanitation, monitor milk quality</td>
</tr>
<tr>
<td>Unclean</td>
<td>Poor quality, off flavoured or old milk, unwanted microbial contaminants, allowing off-flavoured to be ‘aged’, improper Cheddaring technique</td>
<td>Upgrade milk quality, improve sanitation, standardize Cheddaring process</td>
</tr>
<tr>
<td>Whey taint</td>
<td>Poor whey expulsion, improper Cheddaring technique</td>
<td>Standardize Cheddaring process, make sure expelled whey is free to drain away, wash curd 32°C water to remove whey</td>
</tr>
</tbody>
</table>

**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/neurtralized/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

Introduction

The microstructure, or structural arrangements of components, in food products is determined by two factors: processing and composition. The main components in food products are protein, fat, carbohydrates, water, and air. A careful selection of these components, based on their chemical and physical properties, in combination with optimal processing conditions determines a food’s microstructure and product properties including hardness, spreadability, mouthfeel, emulsion stability, appearance, taste perception, and salt. Microstructure is one of the major controlling factors of texture and functional properties of cheese. Cheese microstructure analysis plays an important role in the quality control of the dairy products. Therefore, electron microscopy techniques, to analyze food microstructure under different processing conditions and composition, are crucial for the control and manipulation of product properties. Structural differences between the cheeses are best observed using Transmission Electron Microscopy (TEM) of thin sections. This technique makes it possible to examine the interior of the cheese particles whereas Scanning Electron Microscopy (SEM) shows surfaces. This is useful, too, because surfaces may be formed by breaking (fracturing) cheese particles; the structure of the protein matrix may thus also be observed. Preparation of the cheese specimens for electron microscopy may preserve the fat globules or may remove them. An attempt has been made in this article to include the recent developments in study of microstructure of fat component in cheese.

Development of microstructure of cheese

Milk is the starting ingredient and basis for natural cheese production. Microstructurally, milk is a dispersion of milk fat globules and proteins dispersed in a continuous phase of water, lactose, and minerals. During cheese production, the whey proteins are removed during a dehydration step, and the fat and casein are concentrated six to twelve times, depending on the cheese variety. Acidification or enzymatic hydrolysis coagulates the concentrated casein to form a continuous protein network. Milk fat, existing as globules (0.1-20 μm in diameter), is encapsulated and held within the protein network. Cooling, the final stage in processed cheese production, is a critical step in which the microstructure of the protein and fat matrix are established, ultimately impacting final cheese texture. Fat is known to greatly contribute to the textural characteristics of many dairy products, such as yogurts and cheeses. In milk, fat is in the form of globules ranging
Microstructural Change in Cheese Fat

Karami et al (2008) studied the microstructural changes taking place in fat during the ripening of Iranian Ultrafiltered Feta Cheese. In this study, fat globules in Iranian ultrafiltered Feta cheese (3 to 60 d) were directly observed during the ripening
period by SEM. Individual fat globules and aggregates of fat were easily distinguishable on d 3 and had completely disappeared within 20 d of storage. On d 20, only the fingerprints of the fat globules and pools of free fat in the casein matrix remained. After 40 d of ripening, the texture was homogeneous and no fat globules or fat voids were detected.

**Microstructure of cheese with reduced and substituted fat content**

Propelled and driven by health awareness among consumers, there has been a trend *in vogue* for production of cheeses with reduced or substituted fat content. Hence, it becomes imperative for researchers and technologists to ascertain whether it is possible to develop cheese-like product that resembles texturally the original cheese they seek to substitute. In this context, the study of microstructure appears a viable, visual and state of the art methodology.

Besides change in mineral content in cheese, such as that obtained through condensing of cheese milk, fat also affects body and texture of cheese. The protein matrix, mainly casein that is interlaced with fat gives cheese its smooth, firm, pliable body and texture. In Reduced fat Cheddar Cheese, the body and texture tend to be firm and rubbery because fewer fat globules are dispersed in the protein matrix. Studies have indicated that fat plays a significant role in the microstructure of many full fat and reduced fat hard and semi-hard cheeses. In reduced fat cheeses, fewer fat globules were interspersed throughout the protein matrix although the protein matrix remained relatively smooth and the protein matrix dominated the structural network of cheese. These changes in the protein matrix confirm that cheese composition affects microstructure. The study of microstructure of reduced fat Cheddar cheese manufactured from condensed milk (Anderson & Mistry, 1994) revealed an interrelationship among cheese milk treatments, cheese body and texture scores, and microstructure from the cheeses. Body and texture scores decreased as concentration increased, and microstructure became increasingly rough and dense, with an uneven protein matrix.

In another study on reduced-fat Edam cheese (Tungjaroenchai, 2003) containing mixtures of adjunct cultures during ripening, full-fat control, reduced-fat control, and reduced-fat Edam cheeses containing adjunct cultures (*Brevibacterium linens, Lactococcus lactis ssp. diacetylactis, Lactobacillus helveticus, and Lactobacillus reuteri*) were produced. The level of milk fat in cheese was found to have a significant effect on the protein matrix as determined by SEM. Proteolysis during ripening led to hardening and reduced springiness and chewiness in all cheeses. Mixed adjunct cultures in reduced-fat cheese showed the same chewiness quality as the full-fat cheese. A decrease in cohesiveness was obtained as a result of added mixed adjunct cultures in reduced-fat cheese during ripening. Cheese composition and microbial activity influenced the microstructure of cheeses.
Cheese-like products prepared from vegetable oils-skim milk emulsions in substitution of whole milk can contribute to a healthier saturated/unsaturated fatty acids balance in the diet. Milk proteins and small molecule emulsifiers compete for interfacial sites in oil-in-water emulsions affecting oil droplet size and distribution, which influence the cross-linking of the casein chains and the mechanical properties of these products during heating. Lobato-Calleros (2002) studied the microstructure and texture of Mexican Manchego cheese-like products whose fat fraction was made up by canola oil and different combination of emulsifiers. A full-fat control Manchego cheese (QGL) was prepared from whole milk (30 g fat/L milk) and nine Manchego cheese-like products (QACS) were prepared from skim milk (0.2 g fat/L) added with a blend of canola oil and emulsifiers (30 g/L of skim milk). The lipophilic emulsifiers sorbitan monostearate (S) and glycerol monostearate (G), and the hydrophilic emulsifier polyoxyethylene sorbitan monostearate (P) were used in different concentrations. After 30 days of aging, the cheeses were subjected to Instrumental Texture Profile Analysis and microstructural analysis using SEM. High concentrations of P in the emulsion resulted in cheeses with higher hardness, elasticity and chewiness, but lower adhesiveness. The above-mentioned textural characteristics were associated with the formation of small fat globules surrounded by a dense protein matrix. Contrarily, cheeses with high concentrations of S showed an interrupted low-density protein matrix with larger fat globules than the cheeses with high concentrations of P. These structural characteristics were related to low hardness, elasticity and chewiness, but with high cohesiveness and adhesiveness values. G did not affect cheese texture. These results indicate that it is possible to develop cheese like product that resembles texturally the original cheese they seek to substitute, even though their present different microstructural features.

Further José Velásquez-Varela (2003) extended this study to determine the effect of three commercial emulsifiers blended in different proportions upon the microstructure arrangement and rheological properties during heating of cheese in comparison to the milk fat counterpart. Nine Mexican Manchego cheese-like products (MCLP) were made from canola oil-skim milk emulsions, prepared using different blends of lipophilic and hydrophilic emulsifiers (polyoxyethylene sorbitan monostearate (P), sorbitan monostearate (S) and glycerol monostearate (G)). A control Mexican Manchego cheese (MCFF) was prepared from whole milk. Temperature sweeps between 25 to 70 °C (1°C/min) were obtained at a frequency of 1 Hz and a shear stress of 0.2 kPa in a dynamic shear rheometer. Microstructure analysis was done using Scanning Electron Microscopy. Values of G’ and G″ of the MCFF cheese were the highest compared with those of the MCLP cheeses, but tan values of the MCFF cheese were lowest than those exhibited by the MCLP cheeses over all the range of temperature studied. P contributed to obtain MCLP cheeses having small oil droplets immersed in a compact and dense protein network, which exhibited higher storage and loss modulus values. Predominance of S and G in the
Water-in-oil-in-water (W1/O/W2) multiple emulsions have been proposed as being suitable for developing reduced-fat food products, as these systems comprise an inner aqueous phase that effectively reduces the polyunsaturated fat mass fraction occurring in an equivalent O/W emulsion. Morphology of W1/O/W2 emulsions deviates greatly from that of O/W emulsions, in that they exhibit very small inner water droplets encased in much larger oil droplets. The size and distribution of the W1/O/W2 droplets is affected greatly by the choice of external aqueous phase emulsifier/stabilizer employed. Both, droplet size and the emulsifier/stabilizer utilized may modify the structural properties of the cheeses through their interactions with the other components. Ofelia Sandoval-Castilla et al (2007) investigated the microstructure of white fresh cheese-like products containing multiple emulsion droplets in substitution of milk-fat globules to determine the effect of substituting milk-fat globules by W1/O/W2 emulsion droplets stabilized with different biopolymers on the chemical composition, yield and microstructure of white fresh cheese. Five W1/O/W2 emulsions were prepared at room temperature using a two stage emulsification procedure. In the first stage, a W1/O emulsion having 20 % wt of dispersed aqueous phase (W1), 8 % wt of low-molecular weight emulsifiers, and 71 % wt of canola oil (O) was prepared using a high shear homogenizer. In the second stage the W1/O emulsion (20 % wt) was re-emulsified into five different biopolymers aqueous solutions (80 % wt): gum Arabic (GA)(10 % wt), carboxymethylcellulose (CMC) (0.5 % wt), amidated low-methoxyl pectin (LMP) (2% wt), GA + CMC (10 % wt + 0.5 % wt), and GA + LMP (10 % wt + 2 % wt). The microstructure of the white fresh cheese was found to be altered by the substitution of the milk fat globules by multiple emulsion droplets. The full fat white fresh cheese (WFC) cheese had a fat content that was significantly higher than that of the reduced-fat white fresh cheese-like products (EC) cheeses. The EC\textsubscript{GA} cheese had a significantly higher fat content than EC\textsubscript{LMP} and EC\textsubscript{CMC} cheeses, but similar fat content to those of EC\textsubscript{GA-LMP} and EC\textsubscript{GA-CMC} cheeses. Protein content was inversely proportional to the fat content of the EC cheeses; in contrast the cheeses having higher fat contents exhibited higher yields. The moisture content of the WFC cheese was intermediate between those corresponding to the EC cheeses. The effect of the multiple emulsion droplets on the microstructure characteristics was governed by the type of biopolymer(s) used as emulsifiers/stabilizers. In general terms, the microstructure of the WFC cheese consisted of a relatively compact structure interrupted by numerous milk-fat globules of various sizes (0.7 to 3 μm). The ECCMC cheese was characterized by a highly interrupted protein network, where the emulsion droplets were the smallest 1.5 μm) and were uniformly distributed.
ECGA cheese showed a few interrupted and coarse protein network containing relatively large (6.7 μm) and other smaller spherical emulsion droplets (3.8 μm) covered by strands of fused casein micelles. Probably the hydrophobic polypeptide chains of gum Arabic adsorbed at the oil droplets surface interacted with casein strands of the protein matrix. ECLMP cheese was characterized by a granular protein matrix, containing amorphous slightly elongated shaped structures possibly composed by calcium pectate aggregates. Furthermore, emulsion droplets that exhibited variable sizes (0.7 to 3 μm) appeared within the amorphous structures. LMP forms insoluble complexes with calcium ions present in milk, which entrapped emulsion droplets. When GA was blended with CMC or LMP spherical oil droplets with intermediate sizes to those produced by the individual polysaccharides were observed.

Conclusion

Body and texture reflect the microstructure of cheese and its microstructure is affected by composition and manufacturing practices. So any attempt to further improve on the quality of existing varieties of cheese and development of new products to satisfy expanding consumers’ demands through intervention of alteration of composition or modification of processing conditions is bound to be reflected in a characteristic microstructure. As the majority of elements that critically participate in transport properties, physical and rheological behavior, textural and sensorial traits of foods are below the 100 lm range, the study of microstructure with the help of electron miscopy is poised to be used more frequently in future to assess and evaluate the feasibility of development of tailor made and customized dairy foods.

Suggested reading

- 3rd International Symposium on Food Rheology and Structure, Zürich, Switzerland 569-570.
Introduction

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.

Development of such product will offer value-addition and improve health benefits of milk and milk by-products by combining these with underutilized cereals and applying advanced technologies for their processing and preservation.

Cereal grains constitute a major source of dietary nutrients all over the world. Although cereals are deficient in some basic components e.g. essential amino acids, fermentation may be the most simple and economical way of improving their nutritional value, sensory properties and functional qualities. In the development of raabdi, pearl millet, sorghum and wheat flour was used. The chemical composition and nutritive values of these flours is discussed as under.

Chemical composition and nutritional value of Sorghum and Pearl millet

Cereal crops are energy dense, containing 10000-15000 kJ/Kg, about 10-20 times more energy than most succulent fruits and vegetables. Nutritionally, they are important sources of dietary protein, carbohydrates, the B complex of vitamins, vitamin E, iron, trace minerals and fiber. Like other cereals, sorghum is predominantly starchy. The protein content is nearly equal among these grains and is comparable to that of wheat and maize. It also has the highest production of food energy per unit of human or mechanical energy expended (Exceeding even maize silage, sugarcane, and maize grain. Proximate composition of sorghum and pearl millet is given in table 1.

Starch is the major storage form of carbohydrate in sorghum and millets. It consists of amylopectin, a branched-chain polymer of glucose, and amylose, a straight-chain polymer. With values ranging from 56 to 73 percent, the average starch content of sorghum is 69.5 percent. About 70 to 80 percent of the sorghum starch is amylopectin and the remaining 20 to 30 percent is amylose. In pearl millet, the
starch content of the grain varies from 62.8 to 70.5 percent, soluble sugar from 1.2 to 2.6 percent and amylose from 21.9 to 28.8 percent (Jambunathan and Subramanian, 1988). Lower values for starch (56.3 to 63.7 percent) and amylose (18.3 to 24.6 percent) have been found in some high-yielding Indian pearl millet varieties.

Table 1: Proximate composition of sorghum and Pearl millet grains

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Sorghum</th>
<th>Pearl millet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g)</td>
<td>10.4</td>
<td>11.8</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>3.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>1.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Crude fibre (g)</td>
<td>2.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>70.7</td>
<td>67.0</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>329</td>
<td>363</td>
</tr>
<tr>
<td>Ca (mg)</td>
<td>25</td>
<td>42</td>
</tr>
<tr>
<td>Fe (mg)</td>
<td>5.4</td>
<td>11.0</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>0.38</td>
<td>0.38</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.15</td>
<td>0.21</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>4.3</td>
<td>2.8</td>
</tr>
</tbody>
</table>

The second major component of sorghum and millet grains is protein. The quality of a protein is primarily a function of its essential amino acid composition. Egg and human milk proteins, for their very high biological value, have been considered as reference standards. Sorghum protein differed in its essential amino acid profile. It is a rich source of leucine, valine, phenylalanine and isoleucine but lysine is the most limiting amino acid in sorghum grain (Indira and Naik, 1971). Pearl millet, like sorghum, contains about 9 to 13 percent protein. The essential amino acid profile shows more lysine, threonine, methionine and cystine in pearl millet protein than in proteins of sorghum and other millets. Its tryptophan content is also higher.

The crude fat content of sorghum is 3 percent, which is higher than that of wheat and rice but lower than that of maize. The germ and aleurone layers are the main contributors to the lipid fraction. The germ itself provides about 80 percent of the total fat (Rooney and Serna-Saldívar, 1991). The fatty acid composition of sorghum fat is: linoleic acid 49 percent, oleic 31 percent, palmitic 14 percent, linolenic 2.7 percent, and stearic 2.1 percent. The fat content of pearl millet is similar to that of sorghum and it is highest among the millets.

Sorghum grain is a rich source of phosphorous (352 mg/100 g) and Magnesium (171 mg/100 g). Ca, Fe, Zn, Cu, Mn, Mo and Cr are found in traces. Pearl millet also contains almost equal amount of phosphorous (379 mg/100 g) and Magnesium (137 mg/100g) but it contains higher levels of iron and calcium than sorghum.

Other than these major constituents, sorghum and pearl millet are also rich source of vitamins and dietary fibres.
As with other foodstuffs certain nutritional inhibitors and toxic substances are associated with these cereals. These factors modify the nutritional value of the individual grains and some of them have very serious consequences. But these antinutritional factors can be reduced by germination and fermentation of cereal grains.

**Technology of Raabdi (Sorghum and Pearl Millet based)**

A technology has been developed for manufacturing pearl millet based and sorghum based fermented milk beverage. These products were named as bajra lassi and jowar lassi, respectively.

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 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 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.

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 whenever required.

**Ready-to-reconstitute beverages**

There is a great demand of ready-to-reconstitute (RTR) products including dairy products in India. Efforts were therefore made to develop a technology for production of ready-to-reconstitute sorghum/pearl millet based fermented milk beverage by adopting spray drying method. Milk solids were used in form of concentrated skim milk and cream (CSM), sorghum and pearl millet solids in form of malt. Concentrated standardized milk with different TS levels and pearl millet flour levels were tried. Two cultures viz. NCDC-167 and NCDC-263 were used for
fermentation of mixture having CSM and cereals malt. The optimum levels were decided on the basis of convenience in spray drying, and sensory evaluation and physicochemical properties of the reconstituted beverage. The effect of stage of addition of pearl millet and sorghum solids to milk solids was also studied. For this purpose two stages were followed viz. before fermentation and after fermentation. In the first case cereal malt was added to milk solids before inoculation of culture followed by fermentation. In the second case the culture was added to CSM and fermentation was carried out, then the flour was added to the set curd. The product obtained by the addition of flour to CSM before fermentation stage was found to be more acceptable.

![Flow diagram for manufacturing pearl millet/sorghum based fermented milk beverage](image)

**Figure 1: Flow diagram for manufacturing pearl millet/sorghum based fermented milk beverage**
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 analyzed for gross composition and physicochemical properties. The conditions of reconstitution of powder into beverage were also standardized.

**Extension of shelf life**

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 the trials were undertaken 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 sachet 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.
Figure 2: Flow diagram for manufacturing ready to reconstitute cereal based fermented milk beverage
References

MICROBIOLOGICAL QUALITY OF MILK IN RELATION TO CHEESE MAKING

Surajit Mandal, Rameshwar Singh, R.P. Singh

Introduction

The number and types of micro-organisms present in milk and dairy products at any particular period depended on the microbial quality of the raw materials, the conditions in which the products were produced and the temperatures and duration of storage, feeding of the animals, season, area, using different starter cultures etc. The quality and safety of milk can be affected if proper care is not taken during production, procurement, transportation, processing, storage and distribution, and consumption. Microorganisms multiply milk under ambient conditions and cause spoilages and health hazards. The most common spoilage micro-organisms in milk and dairy products are *Pseudomonas* spp., coliforms, *Bacillus* spp., *Clostridium* spp., lactic-acid producing bacteria, yeasts and moulds, enterococci etc. On the other hand, milk-borne and milk-product borne outbreaks, caused mostly by cheeses, represent 2–6% of the bacterial food-borne outbreaks reported by surveillance systems from several countries. Cheese represents a large risk of bacterial food-borne outbreaks because of pathogen microflora, divided into pathogens of current concern (*Salmonella* spp., *Campylobacter* spp., coagulase-positive staphylococci, *Listeria monocytogenes* etc.), and those which cause disease only occasionally (*Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium botulinum*, *Streptococcus zoepidemicus* etc.). The low storage temperature of milk when coupled with processing technology to produce very low initial bacteria count in fluid milk could produce fluid milk that will maintain flavour quality at low temperatures. The production of clean and wholesome milk and processing under proper conditions are important for better quality of cheeses.

Bacteriological contamination of raw milk

Most the microorganisms are undesirable in milk because they may be pathogenic or produce undesirable bio-transformation of milk components. The pathogenic microorganisms in milk can affect humans or animals. Contamination of the raw milk in an order of magnitude of $10^2$ to $10^3$ microorganisms/ ml is practically unavoidable. This contamination is due to microorganisms present in the interior of the udder or in teat canal. Under condition of careful milking, $10^4$ microorganisms/ ml could be expected. The main contribution of this order of magnitude comes from the microorganisms present at the surface of the teat and in the milking systems. Under appropriate conditions of the storage with cooling to $4^\circ$C, the number of microorganisms can be kept in the range of $10^4$ to $10^5$cfu/ ml. Sensorial detectable
differences occur at total counts between $10^6$ to $10^7$ cfu/ml and are depends on the species and activity of the respective microorganisms. Most of the bacteria present in raw milk are contaminants of the outside and gain entrance into the milk from various sources including soil, bedding, manure, feed, milking equipments, etc.

**Microorganisms in raw milk and significances**

The presence and multiplication of saprophytic and pathogenic bacteria in raw milk might change the milk composition and produce toxins, and influence the quality and safety of the milk and milk products. Moreover, flavour of the raw milk may be adversely influenced and heat-stable bacterial enzymes may continue to act in products particular during long storage and adversely affect the stability milk and milk products. The pathogenic bacteria include “classical” microorganisms and “emerging pathogens”. At present Salmonella, pathogenic *Escherichia coli* strains, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni* are the most important.

According to the main points of attack on the major milk constituents, the saprophytic bacteria are subdivided as follows:

a) Microorganisms degrading milk carbohydrate (lactose) are classified as glycolates, e.g. streptococci, lactobacilli, Coliforms

b) Microorganisms degrading proteins are classified as proteolytes, e.g. pseudomonas, enterobacteriaceae, aerobic spore-formers.

c) Microorganisms degrading lipids are classified as lipolytes, e.g. pseudomonas, micrococi, aeromonas, corynebacteria

The effect of growth of saprophytic bacteria in milk may be important in three ways as follows:

a) The change in milk composition may interfere with manufacture, if fermentation is involved in the manufacture process, and this may affect the yield and quality of the product, e.g. cheese.

b) The flavour of the raw milk may be adversely influenced (e.g. rancidity) and this may directly affect the flavour of the product e.g. cottage cheese.

c) Heat-stable bacterial enzymes may continue to act in the product, particularly during long storage, and adversely affect the stability and/or flavour of cream and UHT milk.

The human pathogens transmitted through milk are classified into food infection and food poisoning groups. In food infection milk act as a carrier of the microorganisms, this enters in human body through milk. It takes time to a person to become ill and fairly small numbers of microorganisms may suffice to cause illness. In food poisoning, preformed toxins in milk are responsible. Consumers rapidly fall ill. Large numbers of the pathogenic microorganisms are usually needed to cause food poisoning.
Effect of microbiological quality of milk on the quality of cheeses

Microbiological quality of cheeses

Most of the cheeses traditionally are made from raw milk at small, artisanal cottage cheese-makers. Using raw instead of pasteurized milk keeps a larger proportion and diversity of strains belonging to endogenous lactic acid flora and secondary flora that may play an important role in the development of many desirable characteristics in cheese, particularly its specific sensory properties. The native, particularly lactic-acid flora, can also be used as protective cultures to inhibit harmful micro-organisms in milk. Ras cheese (hard cheese produced in Egypt) is manufactured under artisan conditions from raw cow’s or mixture of cow’s and buffalo’s milk without using starter cultures and marketed when it has a queried sharp flavour close to kefalotyic cheese after 3 to 6 months. In traditional Ras cheese, coliform persisted to the end of ripening, and yeasts and molds increased through ripening till the end of ripening. Psychrotrophic bacteria were also increased during ripening. High number of coliforms, and yeasts in Tetilla (raw cow milk cheese) and after one month of ripening high number of coliforms, enterococci, aerobic spore-forming microorganisms, yeasts and moulds, proteolytic and lipolytic micro-organisms were present in the cheese. Enterococci were widely distributed in raw milk cheese and were generally thought to positively affect the development of flavour. E. coli, coagulase positive staphylococci (S. aureus and related species) were present in cheese.

Pecorino Siciliano (hard cheese produced in Sicily, Italy) are prepared from raw ewe’s milk without the addition of starter cultures. The ripening is exclusively carried out by the indigenous bacteria population present in milk and in the dairy environment. Raw milk microflora, in particular native lactic acid bacteria, is considered to increase the diversity of flavour in cheeses, and is involved in the production of the typical characteristics of cheese contributing to flavour development. Reports regarding food-borne disease outbreaks involving dairy products and food surveillance indicate fresh cheese as a potential vehicle of food pathogens such as Salmonella spp., Listeria monocytogenes and enterotoxigenic Staphylococcus aureus. The presence of small numbers of Enterobacteriaceae is common in raw milk, but high numbers are indicative of poor husbandry, poor hygiene practices during milk collection or bad preservation. The microbiological quality of “primosale” on retail sale in the street markets of Palermo, Italy was studied by detecting the common food pathogens Listeria monocytogenes and Staphylococcus aureus and indicator microorganisms, such as Escherichia coli, Enterobacteriaceae and Staphylococcaceae. A high contamination of bacteria belonging to Enterobacteriaceae and Staphylococcaceae was found in 42% and 50% of the cheeses analyzed, respectively. Such results indicate poor husbandry and poor hygiene practices during milk collection or preservation or during cheese production processes and handling. Serra da Estrela cheese is manufactured from
raw ewes' milk and curdled with an aqueous extract of the wild thistle *Cynara cardunculus*, without addition of any commercial starter culture. Since no starter culture is added, the native micro-flora plays an important role during cheese ripening. However, microstructural differences were apparent between cheeses manufactured with refrigerated versus non-refrigerated milk. Coagulase-positive staphylococci were detected only in three samples of Manchego cheese, one of these isolates producing enterotoxin A. Staphylococcal thermo-nuclease was also detected in Manchego and Burgos cheese. The aerobic spore-forming bacteria found in cheese came from raw milk, and pasteurisation would likely not cause any reduction in their numbers. Various groups of micro-organisms identified are part of those generally encountered in raw milk cheese.

**Physico-chemical and sensory quality of cheeses**

Milk refrigeration has been tested in attempts to avoid microbial proliferation between the milking and cheese making steps. However, refrigeration raises concerns pertaining to growth and proliferation of psychrotrophic bacteria, which are known to produce very active (and thermostable) proteolytic and lipolytic enzymes. These enzymes catalyze reactions that increase the levels of free fatty acids causing potential off-flavours, and partial solubilization of beta-caseins. This causes a decreased diameter and an increased hydration degree of casein micelles, both of which promote a greater stability. As a result, a less compact and more fragile final coagulum is attained. Milk refrigeration prior to cheese (Serra da Estrela cheese) making appeared to control growth of *Enterobacteriaceae*, and to promote formation of a coarser protein matrix. Although the micro-structural observations did not permit detection of significant differences during ripening, differences were detected in cheeses manufactured from refrigerated milk. These cheeses had a looser matrix, where lactobacilli and yeasts predominated. The higher temperature of the non-refrigerated milk may induce faster acidification by the native bacteria, thus producing a coarser gel structure (which leads to a higher porosity) and favouring whey expulsion. Refrigeration, in turn, enhances microbial-mediated lipolytic activity, thus disrupting the fat globule network with a concomitant decrease in the number of these globules. Cheese yield was affected by initial psychrotrophic populations and length of time raw milk was stored. Recovery of cheese solids is decreased by approximately 0.5% for manufacturing grade milk per day of storage up to 4 d. Further storage increased the loss of cheese solids, and loss of cheese yield correlated with increase in bacterial population. Yield loss appeared to be due to exo-cellular enzymes causing breakdown of proteins and fats. Protein degradation was accompanied by increased moisture in the curd. Cheese quality decreased as psychrotrophic populations increased. Stored milk cheese had a weak body with bitter flavours typical of that produced by heat-stable proteolytic enzymes. Extracellular proteinases from psychrotrophs may cause reduced cheese yields and off-flavors, but the problem does not appear to be significant at
populations <10^6 cfu/ml (Fox, 1989). The long ripened varieties of cheeses have to be manufactured with milk from cows which are not allowed to be fed with silage because of contamination of milk with spores of Clostridium tyrobutyricum causing late blowing in cheese due to butyric acid fermentation, the metabolites of which i.e. butyric acid, CO_2 and H_2 lead to total deterioration of cheese. The effect of storage of raw milk for up to 12 days at 5°C on the yield of cottage cheese was decreased with an increase in time that raw milk was stored. Yield decreases were 2.5 to 3% per day of low temperature storage after the bacterial count of raw milk attained 10^6/ml. These decreases occurred in the first 2 days of added storage of the bulk fluid milk from the local processor. Bacterial analysis of the raw skim milks showed rapid increases in standard plate, psychrotrophic, and proteolytic counts. Coliform count also exhibited a rapid increase, but the final population was not as large as psychrotrophic and proteolytic counts. High level of coliform contamination in cheese milk causes early blowing defects of cheese curd or curd floating defect due to hetero-fermentative end product of lactose (CO_2 and H_2).

**Improvement of quality and safety of cheeses**

The raw milk whose manufacturing process does not involve any heat treatment must only meet a few microbiological standards: the plate count at 30°C should be ≤ 100,000 cfu/ml, low in Staphylococcus aureus, somatic cell count ≤ 400,000/ ml and absence of antibiotics. The microbiological criteria for cheese made from raw milk are the absence of Listeria monocytogenes and Salmonella spp. in 25 g of sample, Staphylococcus aureus and Escherichia coli in low numbers. In Italy, current regulations require unpasteurized cheeses to have at least 60 days of ripening for safety reasons. The 60 day holding period offers an alternative to pasteurization based on the assumption that any bacterial pathogens present in fresh cheese would die within this period and alternatively, the cheese can be produced with thermized milk (57-68°C for at least 15 s). In Manchego cheese and La Serena cheese, the numbers of Enterobacteriaceae dropped to negligible levels by 120 and 150 days of ripening, respectively, in cheeses manufactured from refrigerated and non-refrigerated milk. Heat treatment destroyed all the coliforms and probably other undesirable micro-organisms of raw milk and this improved the microbiological quality of the cheeses. Pasteurisation is the primary means of ensuring that related cheese does not represent a health risk. Still, even industrial pasteurisation cannot guarantee the absence of pathogenic micro-organisms because they are present in large numbers in raw milk or due to post-pasteurisation contamination. However, pasteurisation also reduces a large proportion of lactic acid bacteria and secondary flora that may play an important role in the development of many desirable characteristics in cheese. Starter cultures also affect the cheese yields, moisture content, acidity, FFA and TVS. Lactobacilli were found to be the most important organism by their presence in large numbers in all the cheeses. To avoid the risk of late blowing a technology based on cross flow microfiltration or bactofugation
eliminate of bacteria and spores from milk. However, microbial enzymes pass through and can cause rancidity and flavour and texture defects in cheese. It is therefore important that the initial milk for cheese making is of good microbiological quality. The low bacterial count of cheese milk led to significantly reduced concentration of free short-chain acids in these cheeses. The elimination of indigenous raw milk flora could be partly restored by addition of 1-2% of raw milk. Adjunct cultures such as *L. casei*, *L. helveticus*, *Micrococcus* spp. and *Candida utilis* influenced to some extent the flavour and the proteolysis in cheeses.

**Conclusions**

Milk used for cheese making must be of good microbiological quality, pathogenic bacteria should be absent, psychotropic bacteria have heat resistant lipases and proteases which may reduce yield, but may also cause undesirable flavours in the ripened cheese. Clostridia spores cause late blowing in cheeses with eyes, but also to cheeses with closed texture these bacteria induce inedible flavours. The various stages in the milk processing chain from milking the cow to consumption have to be under control in order to assure the quality and safety of milk and milk products. Adherence of basic good manufacturing practices is one of the first steps to achieve this. HACCP can be applied as a tool to assess hazards and establish control system that focus on preventive measure rather than relying mainly on end product testing. Ensuring of the raw materials is of best quality and elimination of spoilage and pathogenic bacteria from milk by heat treatments and any other suitable means.

**References**


Introduction

Fermentation is achieved through the process of bio-conversion of organic substances by microorganisms and/or enzymes of microbial, animal or plant origin. Fermented foods are important components of diets in many parts of the world, especially Southeast Asia, the Near East and parts of Africa. In many cases in the final products make important contributions to the diet as source of proteins, calories and some vitamins. During fermentation some vitamins increase; others may decrease. Digestibility of fermented foods is improved over the ingredients. 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. Fermented milks are manufactured throughout the world and approximately 400 generic names are applied to traditional and industrialized products.

Types and uses of fermented foods

Fermented foods are commonly used around the world in varying amounts. Different areas of the world have developed fermented foods that improve their diets, especially during times of hardship. Consuming these foods can provide nutritional benefits beyond those of unfermented foods, which is particularly important in areas of the world where malnutrition is prevalent. The fermented are classified based on the microorganisms that dominate in the product, including their principle metabolites and the starting materials. Dairy sources of fermented foods include cheese, kefir and yogurt. Yogurt can be made from cow's milk or goat's milk; goat milk yogurt is said to be easier to digest. Plant sources of fermented foods include kimchi, sauerkraut, miso, tempeh and soybeans. Fermented foods such as bread and wine have been consumed for thousands of years. Probiotics are the dietary supplement form of fermented foods. They are available in tablets, capsules, powders, beverages and suppositories. A variety of foods can be fermented.

Traditional uses: Fermented foods can preserve for long, are easy to digest. Fermentation makes unsafe foods or inedible things edible and makes foods more nutritious. During time of food shortage, things that are not normally eaten can be fermented to make them more palatable. Probiotics are also found in many fermented foods, so these foods can lower the risk for diarrhea. The strong taste of fermented foods is useful in adding flavour to diets that might otherwise be bland.
Nutritional benefits of fermented foods

Fermentation enhances the nutritional value of food through the biosynthesis of essential amino acids, vitamins and protein and also increases the absorption of calcium, B vitamins etc. The action of micro-organisms during the preparation of cultured foods or in the digestive tract has been shown to improve the quantity, availability and digestibility of some dietary nutrients. Fermentation of food with lactic acid bacteria increases folic acid in yogurt, bifidus milk and kefir. Similarly, niacin and riboflavin levels in yogurt are increased with fermentation. Lactic acid bacteria are known to release various enzymes and vitamins into the intestinal lumen. Bacterial enzymatic hydrolysis may enhance the bioavailability of protein and fat and increase the production of free amino acids, short chain fatty acids (SCFA), lactic acid, propionic acid and butyric acid are also produced by lactic acid bacteria. When absorbed these SCFAs contribute to the available energy pool of the host and may protect against pathological changes in the colonic mucosa. SCFA concentration helps to maintain an appropriate pH in the colonic lumen, which is critical in the expression of many bacterial enzymes and in foreign compound and carcinogen metabolism in the gut etc. Several lines of evidence show that the appropriate strain of lactic acid bacteria, in adequate amounts, can alleviate symptoms of lactose intolerance. Streptococcus thermophilus, Lactobacillus bulgaricus and other lactobacilli used in fermented milk products deliver enough bacterial lactase to the intestine and stomach where lactose is degraded to prevent symptoms in lactase non-persistent individuals. Some of the lactic acid bacteria are able to synthesize exo-polysaccharides having probiotic potential e.g. galacto-oligosaccharides, dextran etc. One of the important outcomes of food fermentation is

Prevention of malnutrition: Fermented foods can help prevent malnutrition in three ways. First, fermenting makes more food available overall. Second, the process of fermenting foods increases the amounts of certain vitamins and minerals in foods, including biotin, nicotinic acid, riboflavin, thiamine and vitamin B12. Finally, fermenting some foods makes them easier to digest, breaking down fiber that you wouldn't normally be able to digest and turning it into sugars that you can digest. This increases the amount of calories you get from the food. Lactose is also partially broken down during fermentation, making yogurt easier to consume for those who are lactose intolerant.

Prevention food-borne illness: Foods contaminated with certain types of bacteria can cause diarrhea which leads to malnutrition. Fermenting foods creates conditions that are unfriendly for these types of bacteria, killing off some types and keeping others from contaminating these foods, making fermented foods less likely to cause food-borne illnesses. These foods also tend to have friendly bacteria, or probiotics, which help to prevent these types of infections. These nutrients help to bolster the immune system against other illnesses as well.
the enrichment of food with essential amino acids, vitamins, mineral and bioactive compounds.

The fermentative action of specific LAB stains may lead to the removal of toxic or antinutritive factors, such as galactose from fermented milks to prevent lactose intolerance and accumulation of galactose). Other examples are the removal of raffinose, stachyose, and verbascose from soy to prevent flatulence and intestinal cramps, proteinase inhibitors from legumes and cereals to prevent malabsorption, phytic acid and tannins from cereals and legumes to increase mineral bioavailability, and natural toxins such as cyanogenic glucosides from cassava as well as biogenic amines from traditional fermented foods. Nutraceuticals are food components that, through specific physiological action, contribute to the health of the consumer. Several nutraceuticals from bacterial origin have been added to food. Through strain selection and process optimisation, the activity of LAB can be modified to increase the content of nutraceuticals in fermented foods such as fermented dairy products. As an example, fermented milks can be produced with LAB starter strains that produce high amounts of low-calorie polyols so as to reduce the sugar content. Certain LAB, such as the yoghurt bacteria *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, are able to produce vitamins such as folate. A controlled use of these bacteria may lead to dairy products with increased folate content.

For example, during tempe fermentation, vitamins like niacin and riboflavin are increased by using the starter culture *Rhizopus oligosporus*. Similarly, thiamine and riboflavin increase during fermentation of *idli*, the fermented rice and black-gram (a type of legume) product of India and Sri Lanka. In order to gain strength, ailing persons and post-natal women in the Himalayas consume *bhaati jaanr* extract (a fermented rice food-beverage) and *kodo ko jaanr* (a fermented finger millet product) due to their high calorie content. Pulque is one of the oldest alcoholic beverages and is prepared from the juices of cactus plants in Mexico. The product is rich in vitamins such as thiamine, riboflavin, niacin and biotin. Another important reason to ferment foods is to coax micro-organisms into producing enzymes that also provide very useful services. These enzymes degrade anti-nutritive compounds and thereby make edible, with enhanced flavour and aroma, things that otherwise would be indigestible and/or unpalatable. Bitter varieties of cassava tubers contain a potentially poisonous substance that can be detoxified via lactic acid bacteria, as in *gari* and *fufu*, fermented cassava root products from Africa. Many people suffer from lactose intolerance or lactose mal-absorption, a condition that causes lactose, the principal carbohydrate of milk, to not be completely digested. The cultures used in making yoghurt and curds, contain substantial quantities of ß-D-galactosidase, something that is thought to help alleviate the symptoms of lactose mal-absorption. Yoghurt, as a viscous food, may delay the stomach emptying and that way help lessen lactose intolerant symptoms. Kefir provides supplemental nourishment for pregnant and nursing women. The exceptional nutritional content of Kefir offers a
wealth of healthy benefits to people in every type of condition. More than just beneficial bacteria, Kefir contains minerals and essential amino acids that help your body with its natural healing powers and maintenance functions. The complete proteins in Kefir are partially digested and therefore more easily utilized by the body. Tryptophan, one of the essential amino acids abundant in Kefir, is well-known for its relaxing effect on the nervous system. Because it also offers loads of calcium and magnesium -- both of which are critical for a healthy nervous system -- Kefir in the diet can have a particularly calming effect on the nerves. Rich in vitamin B12, B1, and vitamin K, Kefir is an excellent source of biotin, a B vitamin which aids the body's absorption of other B vitamins, such as folic acid, pantothenic acid, and B12. The many advantages of maintaining adequate B vitamin intake range from regulation of the normal function of the kidneys, liver and nervous system to helping promote healthy looking skin, boosting energy and promoting longevity. Kefir supplies the phosphorus, the second most abundant mineral in our bodies and helps utilize carbohydrates, fats, and proteins for cell growth, maintenance and energy.

**Therapeutic potential of fermented foods**

Many of the fermented products consumed by different ethnic groups have therapeutic values. Some of the most widely known are fermented milks (i.e., yoghurt, curds). Containing high concentrations of pro-biotic bacteria, these can lower your cholesterol level. One fermented food that has received much attention is the viscous, acidic, mildly alcoholic milk beverage produced by fermentation of milk with a particular grain in Eastern European and Middle Eastern countries.

*Kefir* is easily digested and provides the human body with beneficial micro-organisms that contribute to a healthy immune system — a boon to patients suffering from AIDS, chronic fatigue syndrome, herpes and cancer. Traditionally it has been used for the treatment of tuberculosis and cancer. While both Kefir and yogurt are cultured milk products, they contain different types of beneficial bacteria. Yogurt contains transient beneficial bacteria that keep your digestive system clean and provide food for the friendly bacteria that already are present. Kefir actually helps to colonize your intestinal tract -- a feat that yogurt cannot match. Additionally, Kefir contains several major strains of friendly bacteria not commonly found in yogurt: *Lactobacillus caucasus*, *Leuconostoc*, *Acetobacter* species, and *Streptococcus* species. It also contains beneficial yeasts, such as *Saccharomyces kefir* and *Torula kefir*, which help balance the intestinal flora, including promotion of beneficial yeast in the body by penetrating the mucosal lining. The curd size of Kefir is smaller than yogurt, so it's also easier to digest, making it an ideal food for babies, the elderly, and anyone with digestive health concerns. Kefir is easily digested and provides beneficial micro-organisms that contribute to a healthy immune system. Kefir, which means 'feel good" in Turkish, is an ancient cultured, enzyme-rich food filled with friendly micro-organisms that help balance your "inner ecosystem" to maintain optimal health and strengthen immunity. When used regularly, the
naturally occurring bacteria and yeast in Kefir combine symbiotically to help balance your intestinal flora and boost your immunity. Among its many beneficial powers, contributes to your healthy immune system, promotes a relaxing effect on the nervous system and benefit many who seek a restful night’s sleep, helps support your normal intestinal tract function, promote bowel movements and your healthy digestive system and is beneficial after the use of antibiotics to restore balance to the digestive tract. Another naturally fermented dairy product, Koumiss, found in the Caucasus region, is used in the treatment of pulmonary tuberculosis.

Antioxidant and other properties are reported in foods such as natto, a sticky dish high in protein and popular at breakfast in Japan. Other widely-eaten types of health-boosting sustenance include fermented soybean products found around Asia. Antioxidant and other properties are reported to exist in foods such as natto, a sticky dish high in protein and popular at breakfast in Japan that may help prevent people from having brain haemorrhages. Natto is also rich in vitamin K2, which stimulates the formation of bones and might help to prevent osteoporosis in older people. Similarly, consumption of Indonesia’s tempe reduces cholesterol levels and, like China’s douchi, lowers high blood pressure. China’s douchi lowers high blood pressure. Korea’s famous accompaniment kimchi, may take the prize for most benefits however, reportedly helping to prevent constipation and colon cancer and reduce serum cholesterol, as well as possessing anti-stress effects and the ability to ameliorate depression, osteoarthritis, liver disease, obesity and atherosclerosis. In the Himalayas, a fermented leafy vegetable product called gundruk and a fermented radish tap-root (sinki) have large amounts of lactic acids, ascorbic acid, carotene and dietary fibre, which have anti-carcinogenic effects.

The health benefits of probiotics have been mostly studied in milk based products fermented with Lactobacillus and Bifidobacteria. These products contain a great many chemical compounds depending on the type of milk used (usually cow’s, ewe’s milk or goat milk), on the type of microorganisms added (and their specific biochemical activities) and on the manufacturing process employed. They are characterized by lower levels of free amino acids and certain vitamins than non-fermented milks. Lactobacillus acidophilus and Bifidobacteria have also reported to synthesize folic acid, niacin, thiamine, riboflavin, pyridoxine and vitamin K which are slowly absorbed by the body. The vitamins of the B-complex are frequently obtained as natural ingredients in foods, so addition of L. acidophilus to the diet will more effectively help to meet those requirements. The bio-availabilities to the host of such minerals as calcium, zinc, iron, manganese, copper and phosphorus may also be enhanced upon consumption of fermented dairy products and improve the digestibility of the proteins. The proteolytic system of LAB can contribute to the liberation of health-enhancing bioactive peptides from milk. The latter may improve absorption in the intestinal tract, stimulate the immune system, exert antihypertensive or antithrombotic effects, display antimicrobial activity, or
function as carriers for minerals, especially calcium. Health effects of such oligosaccharides are ascribed to their low-calorie character, their fibre-like nature, and their bifidogenic effect.

Table 1: Potential health and nutritional benefits of functional foods prepared with probiotic bacteria.

<table>
<thead>
<tr>
<th>Beneficial effects</th>
<th>Possible cause and Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved digestibility</td>
<td>Partial break down of proteins, fats and carbohydrates.</td>
</tr>
<tr>
<td>Improved nutritional value</td>
<td>Higher levels of Vit.B and certain free amino acids, viz., methionine, lysine and tryptophane</td>
</tr>
<tr>
<td>Improved lactose utilization</td>
<td>Reduced lactose in product and further availability of lactose</td>
</tr>
<tr>
<td>Antagonistic action towards enteric pathogen</td>
<td>Disorder such as functional diarrhea, mucous colitis, ulcerated colitis and antibiotic colitis controlled by</td>
</tr>
<tr>
<td>Colonization in gut</td>
<td>Survival in gastric acid, resistant to lysozyme and low surface tension of intestine, adhesion to intestinal mucosa, immune modulation.</td>
</tr>
<tr>
<td>Anti-carcinogenic effect</td>
<td>Conversion of potential procarcinogens into less harmful compounds. Inhibitory action towards some types of cancer, reduction of carcinogens promoting enzymes and stimulation of the immune system.</td>
</tr>
<tr>
<td>Hypocholesterolemic effect</td>
<td>Production of inhibitors of cholesterol synthesis. Use of cholesterol by assimilation and precipitation with deconjugated bile salts.</td>
</tr>
<tr>
<td>Immune modulation</td>
<td>Enhancement of macrophages formation, stimulation of production of suppressor cells and γ-interferon.</td>
</tr>
<tr>
<td>Vaccine vehicle</td>
<td>Naturally occurring or rDNA vaccinal epitopes.</td>
</tr>
</tbody>
</table>

Table 2: Commercially available therapeutic milk products and their health claims

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Products</th>
<th>Health claims</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Actimel</td>
<td>Reinforces your natural resistances</td>
</tr>
<tr>
<td>2</td>
<td>Yakult</td>
<td>A healthy start to everyday</td>
</tr>
<tr>
<td>3</td>
<td>Actimel cholesterol control</td>
<td>Helps, if taken regularly as part of a healthy and varied diet, to reduce your cholesterol values</td>
</tr>
<tr>
<td>4</td>
<td>BIO Aloe Vera</td>
<td>Feeds and hydrates in a very self – evident Way : from inside out</td>
</tr>
<tr>
<td>5</td>
<td>Fyos</td>
<td>Promotes a healthy gut balance”</td>
</tr>
<tr>
<td>6</td>
<td>Fysiq</td>
<td>Contributes to a healthy cholesterol level</td>
</tr>
<tr>
<td>7</td>
<td>Silhouette Plus</td>
<td>Soluble Bifidogenic fibers help to preserve and stabilizes the balance of gut flora”</td>
</tr>
</tbody>
</table>

Conclusions
The action of micro-organisms during the preparation of cultured foods or in the digestive tract has been shown to improve the quantity, availability and digestibility...
of some dietary nutrients. Although fermented foods are marketed globally as health foods, functional foods, therapeutic foods, nutraceutical foods or bio-foods, due to urbanization, changes in life-style, and the shifting from traditional food habits to commercial fast foods over traditional fermented foods.

References

TECHNOLOGICAL ADVANCES IN THE MANUFACTURE OF MISTI DAHI

P. Narender Raju, Ashsih Kumar Singh and Dharam Pal

Introduction

*Misti dahi*, also called as *misti doi* or *Lal dahi* or *Payodhi*, is a popular product in Eastern India, mainly West Bengal and partly in Assam, Bihar and Orissa where it is served with the meal accompanied by sweets. It has creamish to light brown colour, firm consistency, smooth texture and pleasant aroma. It is a delicacy of choice during religious festivities and is considered an auspicious item to serve while starting a journey, or any important work. In combination with *rasogolla*, another popular Bengali sweet, *misti dahi* is regarded an extra special dessert on ceremonial occasions both in rural and urban areas of Bengal. In India standards have not been prescribed by the statutory authorities for *misti dahi*. However, the definition of *dahi* as per PFA rules allows the usage of cane sugar and thus, can be adopted for *misti dahi*. Traditionally, *misti dahi* is prepared from cow or buffalo or mixed milk. It is first boiled with a required amount of sugar (6 to 25%) and partially concentrated to about 60 to 70 percent of the original volume by simmering over a low fire (60-70°C) during which milk develops a distinctive light cream to light brown caramel colour and flavour. This is then cooled to ambient temperature and cultured with sour milk or previous day’s *dahi* (culture). It is then poured into earthen vessels of consumer or bulk size and left undisturbed overnight for fermentation. When a firm body curd has set, it is shifted to a cooler place or preferably refrigerated. The product has a characteristic caramelized flavour and a firm body free from holes and whey pockets. Use of earthen vessel absorbs any whey that may tend to separate during storage and distribution. *Misti dahi* is normally served in chilled form. Large variations have been reported in the quality of *misti dahi* sold in different markets (Ghosh and Rajorhia, 1987; Sarkar, et. al., 1996). As a result, successful attempts have been made to develop an industrial method of manufacture of *misti dahi* by Ghosh and Rajorhia (1990a). With technological advances in the processing and packaging of milk, *misti dahi* is now sold in consumer attractive single serve disposable cups by various leading brand owners such as Mother Dairy, Sudha, Visaka, etc. With the growing awareness among consumers about the relation between diet and disease, there is a need to formulate *misti dahi*, conventionally a high fat and high sugar containing product, to cater to the needs of the calorie conscious and targeted population in the present health foods regime. Some of the recent work carried out in this direction has been reported in this article.
Improvements in the manufacturing

Standardization of milk

Different workers have used different milks for the manufacturing of misti dahi. Ray and Srinivasan (1972) used fresh cow milk (5% fat) that was standardized with non-fat dry solids to have total solids content of 16 percent. Ghosh and Rajorhia (1990a) used fresh buffalo milk (5% fat) standardized to contain 13 percent milk solids-not-fat (MSNF) using concentrated skim milk. Gupta, et al., (2000) reported the use of buffalo milk containing 6% fat and 9% MSNF. Goel (1998) has suggested that buffalo milk (6% fat) with 15% MSNF can be used for the commercial production of misti dahi.

Homogenization

It was reported that homogenization of milk at a pressure of 5.49 MPa and temperature of 65°C resulted in production of misti dahi that received a highest flavour score. Increasing pressures from 5.49 to 8.24 MPa did not make any significant differences in flavour scores of misti dahi (Ghosh and Rajorhia, 1990a). The average initial acidity and pH of milk and the resulting product were not affected by the different homogenizing pressures.

Addition of cane sugar

Sugar is added to misti dahi to give it a sweet taste, and also when heated along with milk it gives characteristic caramel colour and flavour. Sugar is added in the form of cane sugar, sugar caramel, or even fresh palm jaggery (gur). Aneja, et al. (2002) suggested the use of corn sugar, corn syrup, maltose, or beet sugar. The content of sugar varied in methods reported by different authors. Ray and Srinivasan (1972) reported the use of 6 percent sucrose while Ghosh and Rajorhia (1990a) reported 14 percent cane sugar usage. Sarkar, et al., (1992a) reported the use of 18 percent sugar. Recently, Gupta, et al., (2000) reported that cane sugar can be used from 16 to 20 percent. Goel (1998) proposed the use of 8 percent sugar along with flavour syrup (5%).

Colour and flavour development

Heating of milk containing sugar develops caramel colour and flavour. But the intensity of the desired colour and flavour depends on the temperature and duration of heat treatment. Ghosh and Rajorhia (1990a) reported that heating to 85°C for 10 min gave desired colour and flavour, while Sarkar, et al., (1992a) reported that 30 min heating at 85°C was necessary. Gupta, et al., (2000) reported that open pan concentration (boiling) of milk to $\frac{3}{4}$th of the original volume along with sugar developed the colour of the mix. In a novel approach suggested by Goel (1998) for the commercial production of misti dahi, flavour syrup at a rate of 5 percent along with 8 percent of sugar before heat treatment gave typical characteristic flavour and colour to misti dahi. However, with a view to make colour and flavour development
a simple, time and energy saving process for industrial applications, we have successfully tried using caramel powder in our laboratory for misti dahi (Raju and Pal, 2009; 2011a; 2011b).

**Starter cultures**

In one of the earliest studies, Ray and Srinivasan (1972) used *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in the ratio of 3:1 at a level of 3% for the production of misti dahi in controlled conditions. It was incubated at 42°C for 4 to 8 hr. However, Sarkar, *et al.*, (1992c) recommended the use of *Streptococcus thermophilus* and *Streptococcus lactis* as starter culture for misti dahi. Recently, a misti dahi based functional food, called misthi yoghurt, containing probiotic cultures such as *Lactobacillus bulgaricus* and *Bifidobacterium bifidus* and prebiotics has been developed by Natarajan and Neethu (2005). Ghosh and Rajorhia (1990a) reported the use of polystyrene cups (100 mL) for packaging of misti dahi. According to these workers the concentrated and sweetened milk inoculated with starter culture is filled aseptically into pre-sterilized containers before incubation. Once filled with the mix, the container is heat sealed with appropriate lids and kept in undisturbed conditions for setting and to yield misti dahi.

**Packaging**

Traditionally misti dahi is packaged in earthen pots. Ghosh and Rajorhia (1990a) reported the use of polystyrene cups (100 mL) for packaging of misti dahi. According to these workers the concentrated and sweetened milk inoculated with starter culture is filled aseptically into pre-sterilized containers before incubation. Once filled with the mix, the container is heat sealed with appropriate lids and kept in undisturbed conditions for setting and to yield misti dahi.

**Storage and shelf life**

Once the desired level of acidity (0.7% as lactic acid) is developed, the set misti dahi is shifted to a cold store or a refrigerator with minimum disturbance and stored at a temperature of 4-5°C. The shelf life of misti dahi packed in glass cups was 48 hr at 30°C and 12 days at 5±1°C where as in earthen pots it was similar to glass cup samples at 30°C but had only 8 days at 5±1°C. However, storage in earthen pots
Reduced fat misti dahi

With the changing lifestyle and dietary patterns, non-communicable diseases like obesity, diabetes, cardiovascular diseases and cancer have become major health problems worldwide. The World Health Organization estimated that worldwide approximately 1.6 billion adults (age 15+), at least 400 million adults and 180 million people were overweight, obese and diabetes, respectively in the year 2005 (WHO, 2006). 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 (Beaglehole and Yach, 2003). However, there are large disparities in cardiovascular disease mortality in different Indian states (Gupta et al., 2006). 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). Calorie conscious people need to achieve a negative energy balance to maintain ideal body weights by cutting down their caloric intake. With a view to cater to the needs of calorie conscious people, successful attempts were made to develop reduced fat misti dahi from buffalo milk (Raju and Pal, 2009). The method described by Ghosh and Rajorhia (1990a) was followed with slight modification for the production of misti dahi. The modification includes use of SMP instead of condensed skimmed milk for standardization of MSNF and addition of caramel powder. The effect of reduction of milk fat, by keeping the total milk solids constant, was studied on the physico-chemical, sensory and textural properties of buffalo misti dahi. Fresh raw buffalo milk was divided into three batches and was standardized to three different levels of milk fat viz. 1.5%, 3.0% and 5.0% using fresh raw skimmed buffalo milk and the total milk solids (TMS) content was adjusted to 18.0% using skimmed milk powder. The batch containing 5.0% fat was considered as control. Acidity, whey separation, viscosity, water activity ($a_w$), lightness ($L^*$), redness ($a^*$), yellowness ($b^*$), firmness, stickiness, work of shear and work of adhesion were determined. Sensory evaluation was carried out using 9-point hedonic scale method. Significant differences ($p<0.05$) were observed in the flavour, body and texture and overall acceptability of misti dahi, that with 3.0% fat being better than 1.5% fat product. Acidity increased with the decrease in fat. Fat reduction did not cause any significant changes in the $L^*$, $a^*$ and $b^*$ values and water activity of misti dahi. The firmness values of misti dahi with 1.5% fat were higher than that of 3.0% fat but the difference was not significant. The stickiness value of misti dahi with 1.5% fat was more than the control but the difference was not significant. Hence, on the basis of above results it was concluded

Misti dahi with enhanced health attributes

Reduced fat misti dahi

caused shrinkage of the product (Ghosh, 1986). Attempts were made in the author's laboratory to extend the shelf life of misti dahi up to using permitted class II preservative.
that highly acceptable reduced fat misti dahi can be produced with 3.0% fat and 15.0% milk solids-not-fat (MSN) (Raju and Pal, 2009).

**Artificially sweetened misti dahi**

India has the largest diabetic population and one of the highest diabetes prevalence rates in the world (King, et.al. 1998; Bjrok, et. al. 2003). Type-2 diabetes is a chronic progressive disease that requires lifestyle changes (Knowler *et al.*, 2002), the key lifestyle interventions being physical activity and a nutritional plan with reduced caloric intake (Franz, 1997). The discovery of a great number of sweeteners has triggered the development of new sugar-free dairy products with alternative sweeteners rather than sugar such particularly for diabetics, people on special diets and/or the obese. In India, most of the traditional dairy products contain high fat and high sugar (Pal and Raju, 2007). Being aware of the impact of sugar on health, today’s health conscious consumer is looking for the low-sugar or sugar-free Indian traditional dairy products. With a view to provide the pleasure of ethnic misti dahi to people on restricted diet, attempts have been made to develop various artificially sweetened misti dahi (ASMD). Artificial sweeteners (binary blend of aspartame and acesulfame-K) and bulking agents (sorbitol, maltodextrin and polydextrose) were used to completely replace cane sugar and maintain the bulk in misti dahi. The relative sweetness of artificial sweeteners and bulking agents were considered for formulating various batches. It was found that among the studied bulking agents, maltodextrin increased acidity, water activity and viscosity of ASMD while decreased syneresis compared to other bulking agents (Raju and Pal, 2011a). Instrumental surface colour (CIELAB) values of ASMD were similar to control sample. Maltodextrin increased hardness, adhesiveness and gumminess values of ASMD compared to sorbitol and polydextrose. Maltodextrin was found to be the most suitable bulking agent in the preparation of ASMD using aspartame and acesulfame-K. However, one has to keep in mind that maltodextrin gets metabolized in human intestines in a similar manner to cane sugar. Hence, alternative strategies have to be framed for recommending maltodextrin containing artificially sweetened misti dahi for diabetic subjects.

**Fiber fortified misti dahi**

With the growing awareness of the link between diet and health among consumers, the demand for dietary fiber fortified health-based traditional Indian dairy products, without compromising the taste and texture, is increasing (Pal, 2008). Dietary fiber promotes beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation. Most dietary fiber comes from plant products such as cereals, roots, tubers, fruits, vegetables, legumes, nuts, and other whole grains. Milk and most milk products are devoid of dietary fiber. With a view to enhance the health attributes of misti dahi and improve marketability, three commercial dietary fiber preparations (inulin, soy fiber and oat
fiber) were incorporated and their effect on the product’s quality in terms of physico-chemical, sensory and textural quality was assessed by Raju and Pal (2011b). Among the three dietary fibers, inulin significantly decreased viscosity and instrumental firmness and increased lightness (L*), redness (a*), yellowness (b*), syneresis and work of shear values of FFMD. Oat fiber settled at the bottom and gave a poor appearance. Soy fiber did not affect the flavor of FFMD. Although overall acceptability scores of inulin and soy fiber containing FFMD were significantly lower than control, they were still above the minimum acceptable score. It was concluded in the study that acceptable quality FFMD could be prepared using inulin and soy fiber Raju and Pal (2011b).

**Dietetic misti dahi**

Based on the outcome of the research work carried out earlier (Raju and Pal, 2009, 2011a, 2011b), variables like milk fat, maltodextrin (bulking agent) and inulin (dietary fiber) were identified as critical factors for partial milk fat reduction, complete sugar replacement and dietary fiber fortification in misti dahi to enhance its health attributes (dietetic misti dahi). The experiments were carried out using central composite rotatable design (CCRD) in realistic vicinity to locate the true optimal values of multiple compositional variables. Besides optimization, the combined effect of these variables on various sensory, textural, colour and physico-chemical properties was demonstrated. Second order polynomials were developed by multiple regression technique for each of the response using a software package, Design Expert (Version 7.0.1). The optimized dietetic misti dahi formulation was validated for its health benefits in diabetes induced animal models.

**Conclusion**

In the current health foods regime, consumers are demanding conventional traditional dairy products with enhanced health attributes. With advances in functional foods and nutraceuticals various functional ingredients have been incorporated into traditional fermented dairy products to enhance their health benefits. Misti dahi is a fermented dairy product of eastern India. With the availability of technology for the industrial production, its popularity is growing across the country as evidenced by its manufacture and distribution leading brand owners. However, its successful marketing in the present health foods regime could be hampered due to its high fat, high sugar and absence of dietary fiber. As a result of recent R&D efforts, now technologies are available for the production of reduced fat, artificially sweetened, fiber fortified and dietetic misti dahi. Misti dahi, a fermented dairy product with inherent therapeutic properties, could be manufactured with enhanced health attributes by technological interventions to suit the palate of health conscious Indian gourmets.
References


Introduction

Aroma and flavour belong among the most important food quality criteria. They are major attributes that influence the selection and consumption of food. Milk and dairy products contain a complex mix of compounds that contribute to the aroma and flavor profile characteristic for each product. During the ripening of most cheese varieties complex chemical conversions occur within the cheese matrix. Cheese flavour results from the breakdown of milk proteins, fat, lactose, and citrate due to enzymes from microorganisms, coagulants, and milk. Many volatile compounds are potentially involved in cheese flavour: such as acids, alcohols, carbonyl compounds, esters, lactones, furans, nitrogen-containing compounds, sulphur and phenolic compounds, hydrocarbons and terpenes. Many of these volatile compounds contribute to the flavour sensation experienced by the consumer. In most cheese varieties breakdown of protein is the most important flavour development pathway. Mould ripened cheese varieties are characterized by extensive lipolysis, thereby such cheese has flavour and aroma contributed both by proteolysis and lipolysis. A very wide spectrum of volatile compounds has been isolated from cheese, in excess of two hundred in some cheese varieties. It is now generally accepted that there is no individual compound which defines cheese flavour completely and that the flavour sensation is the result of numerous compounds present in the correct proportions. This has become known as the "Component Balance Theory". Today, sophisticated and sensitive analytical tests are capable of detecting, identifying and quantitating the specific chemical agents responsible for flavours and these new analytical techniques are so powerful that they can often accomplish this with speed, accuracy and reliability which is not possible using sensory analysis alone.

Instrumental analysis

GC is a form of partition chromatography in which the separation takes place between the stationary phase (a film coated on a solid support) and the mobile phase (a carrier gas) flowing over the surface of the film in a controlled fashion. Because of their superior separation efficiency and versatility, GC methods are the most commonly used analytical techniques in flavor research. GC has tremendous separating power, sometimes in excess of 200,000 theoretical plates per column. This attribute is essential for the separation of complex flavour isolates. Using mass
spectrometry as the detector for GC analysis, allows for identification of chromatographic peaks that elute from the column.

Mass spectrometry (MS) is a form of spectroscopy in which the molecule is exposed to high-energy electrons and through a sequence of steps is broken down into unique charged molecular fragments. The uniqueness of this process allows the method to be used for identification/confirmation of an unknown compound with a sensitivity of 10-100pg. MS is generally used in the flavour area either to determine the identity of an unknown or to act as a mass selective GC detector. GC-MS is an analytical technique used to identify/confirm the identity of compounds as they elute from the GC column and has proven to be one of the most useful analytical techniques for studying volatile and semi volatile odour active chemicals in dairy products. The volatile and semi volatile compounds, in the headspace, are of interest because they can travel to the nose during eating and stimulate the olfactometry receptors in the nasal cavity.

Mass spectrophotometers may be classed as low resolution (LR) or high resolution (HR) instruments. The LR instruments provide mass measurements to the closest whole mass unit, but do not provide elemental composition. High resolution instruments provide sufficiently accurate mass measurements to permit determination of elemental composition. In addition to MS detectors, flavor chemists sometimes employ extremely sensitive detectors for specific classes of compounds. One example is the pulsed flame photometric detector (PFPD) for sub‐ppb measurement of organic sulfur compounds, chemicals that often have extremely low odour threshold detection levels.

The determination of the chemical(s) responsible for flavour in a sample usually involves three steps: preparing the sample for analysis, injecting the sample (or usually an extract of the sample) into the GC-MS and data processing. In addition, many analytical systems are now used by flavor chemists to incorporate an olfactometry detector. With this method, the effluent that elutes from the end of the analytical GC column is split, with a portion of the flow going to the MS detector and a portion going to an olfactometry detector (OD), which is often referred to as a sniff port. While some of the sensitivity of the MS detector is lost, an important advantage is gained: The analyst can sniff each peak as it elutes from the column and determine its odour characteristics. By using GC-MS-OD, the flavor chemist is able to determine the identity, concentration, odour characteristics and odour intensity of each chromatographic peak.

**Sample preparation: A key step in chemical analysis of cheese**

It is usually not possible to directly inject a food sample into a GC without performing some sample preparation. Proteins, fats, complex carbohydrates and other nonvolatile chemicals will degrade in the heated GC injector, resulting in the formation of numerous artifact peaks that can degrade column performance and
Solvent extraction and distillation

Solvent extraction commonly involves the use of pentane, dichloromethane, diethyl ether or some other volatile organic solvent. This limits the method to the isolation of fat-free foods unless an additional procedure is employed to separate the extracted fat. W. Engel et al. (1999) developed a new distillation unit, called solvent assisted flavor evaporation (SAFE), for the extraction of flavor volatiles from complex aqueous matrices, such as beer, fruit juices, milk and cheese. The distillation vessel and “transfer tubes” are thermostated at low temperatures (20°-30°C) to avoid condensation of compounds with high boiling points, and the sample is added by dropping aliquots from the funnel into the vessel to reduce time of extraction. This new method allows for the use of solvents other than diethyl ether and dichloromethane, and it could be used for extracts containing large concentrations of fat. Another advantage of the SAFE technique is that recovery of really authentic flavor—i.e., a flavor sample with organoleptic properties as close as possible to the natural product—is possible. Solvent extraction methods have disadvantages. Large volumes of solvent must be evaporated while retaining the volatile flavor components. Another problem is that sample preparation is time consuming; only one or two samples can be extracted per day.
**Dynamic headspace**

With dynamic headspace techniques, the food sample, which is normally heated to 40° - 60°C, is purged with helium gas. Instead of allowing the sample volatiles to come to equilibrium, the atmosphere around the sample material is constantly swept away by a flow of carrier gas, taking the volatile analytes with it. The volatiles that are swept away are directed to a trap (commonly Tenax), where they are collected and stored until the end of the purging cycle is reached and the trap is

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**Static headspace**

If a complex material, such as milk, yoghurt or cheese, is placed in a sealed vessel, some of the more volatile compounds in the sample matrix will leave the sample and pass into the headspace around it. If the concentration of the volatile compound reaches about 1 ppm in the headspace, it may be assayed by a simple injection of an aliquot in the vessel. How much compound enters the headspace depends on several factors, including its concentration in the original sample, the volatility of the chemical, the solubility of the chemical in the sample matrix, the temperature of the vessel and how long the sample has been inside the vessel. In practice, the food sample is placed into a headspace vial, sealed and warmed to enhance vaporization of the volatiles and incubated for a period of time to establish equilibrium at the incubation temperature. Once the volatiles have equilibrated, an aliquot of the headspace gases is withdrawn with a syringe and injected into the GC. As an alternative, the equilibrated headspace may also be allowed to pass through a sample loop of known volume, which is subsequently flushed into the injection port.

Static headspace methods eliminate the large solvent peak, which may obscure important odour-active analytes. Static headspace is a relatively rapid technique that is easily automated, making it attractive for sample screening applications. The combination of careful monitoring of temperature and equilibrium time, pressure control of the sample loop and automatic injection provides increased reproducibility over manual attempts at headspace analysis and reduces labour costs. Additional advantages include low cost per analysis, simple sample preparation and the elimination of reagents. Relatively poor sensitivity compared to other types of sample preparation techniques is a disadvantage of static headspace method. The maximum temperature for most food products is less than the boiling point of water. Analysis at this fairly low temperature limits the usefulness of the technique for analytes with boiling points over approximately 130°C. Many materials that may be extracted with solvents may elute well at higher GC column temperatures but will be poorly represented in a static headspace chromatogram. Also, reproducibility depends on analyzing a sample after it has reached equilibration, and the time required to achieve this point may, especially for less volatile compounds, be a drawback for some analyses.

**Dynamic headspace**

With dynamic headspace techniques, the food sample, which is normally heated to 40° - 60°C, is purged with helium gas. Instead of allowing the sample volatiles to come to equilibrium, the atmosphere around the sample material is constantly swept away by a flow of carrier gas, taking the volatile analytes with it. The volatiles that are swept away are directed to a trap (commonly Tenax), where they are collected and stored until the end of the purging cycle is reached and the trap is
ready to be desorbed onto the GC column. By removing the volatiles in a continuous fashion, more molecules of the volatiles in the sample are collected for analysis, greatly improving the sensitivity of the test. (Note: In general, the term “purge-and-trap” is used to refer to liquid samples analyzed by bubbling the carrier gas through the liquid, while “dynamic headspace” is used when the sample material is a solid.)

Dynamic headspace is significantly more sensitive than static headspace. Compared to solvent extraction techniques, it offers the advantages of no solvent to evaporate, no interfering solvent peaks in chromatograms and relatively simple automated sample preparation. The disadvantages include more complicated instrumentation. Instrumentation must monitor several steps, valving, heated zones, etc. Instrumentation is more expensive than static headspace instrumentation. Because of complex functioning of the instrument, there are many opportunities for malfunction, including heater damage, valve leaking, contamination and cold spots. Compared to static headspace, dynamic headspace techniques require a little more time per sample (for purging, trap drying and trap transfer, all of which typically require approximately 15 min). However, the technique is much faster than most solvent extraction techniques.

(c) **Solid-phase microextraction (SPME)**

SPME uses a short, thin, solid rod of fused silica (typically 1 cm in length with an outer diameter of 0.11 mm) coated with an absorbent/adsorbent polymer. The coated fused silica (the SPME fiber) is attached to a metal rod, and both are protected by a metal sheath that covers the fiber when it is not in use. The assembly is placed in a fiber holder. The system is a modified syringe. Two sampling methods can be used with SPME depending on the placement of the fiber relative to the sample—immersion or headspace sampling. For dairy products, which contain high levels of fat, carbohydrate and protein, the headspace technique is preferred. In SPME headspace analysis, a fiber is placed in the headspace above the sample. For example, when analyzing volatiles in a milk sample, 3 mL of milk can be placed in a 9 mL glass GC vial containing a small stirring bar and sealed with a septum closure. The sample is then heated (e.g., to 50°C). The fiber is then exposed to the headspace gases for 10 - 30 min, depending on the sample matrix and the analytes of interest. After sample exposure time has elapsed, the fiber is retracted into the needle assembly and removed. The extracted volatiles are thermally desorbed from the fiber in the heated GC injector and transferred to the GC column for separation and analysis. Several types of fibers with varying affinities for specific classes of compounds are available. SPME is particularly well-suited to the analysis of dairy products. The technique is capable of extracting a broader range of analytes than is possible with other headspace techniques. For example, SPME is capable of ppb detection levels for both low molecular weight, highly volatile compounds like acetaldehyde, dimethyl sulfide, acetone and 1,3-pentadiene, as well as high molecular weight, high-boilingpoint compounds like vanillin, lactones and dodecyl
aldehyde. Furthermore, it can be used for quantitating free fatty acids (C4 through C14) in dairy products. This important class of flavor compounds can be particularly challenging and time consuming to extract by other techniques.

**Incorporating the nose in chemical analysis**

The application of new and improved volatile extraction techniques prior to GC-MS in conjunction with modern, sensitive bench top GC-MS instruments often results in dairy sample chromatograms with 100 or more peaks. Unfortunately, the relevance of each peak to a sample’s flavour or OF is not easy to evaluate. One of the major problems in aroma research is to select those compounds that significantly contribute to the aroma of a food. In general, the aroma of a food consists of many volatile compounds, only a few of which are relevant to odour and flavour. A first essential step in aroma analysis is the distinction of the more potent odorants from volatiles having low or no aroma activity. GC in combination with olfactometric techniques (GC-O) is a valuable method for the selection of aroma-active components from a complex mixture. GC-O is a way for flavor chemists to incorporate the sense of smell into their chemical analysis. GC-O is now accepted as one of the most powerful ways to give sensory meaning to the long lists of volatiles appearing in sample chromatograms. GC-O consists of experiments based on human subjects sniffing GC effluents. Experience shows that many key aroma compounds occur at very low concentrations; their sensory relevance is due to low odor thresholds. Thus, the peak profile obtained by GC does not necessarily reflect the aroma profile of the food—that is, sometimes the largest chromatographic peaks in a food extract have the least amount of aroma impact on the food, while the smallest peaks may have the most significant impact. In general, it is very difficult to judge the sensory relevance of volatiles from a single GC-O run. Several techniques are in use to help with this problem. This is based on successive dilutions and GC-injection of a flavor extract, until the assessor no longer detects the odour at the sniffing port. For each GC-elution, the assessor presses a button during the perception of odours to generate individual olfactograms (or aromagrams) made of a series of square signals. After data treatment, a computer generated global olfactogram assigns greater importance to odour peaks that are smelled in the highest dilution of the extract.

**Conclusion**

The advent of new, sensitive and rapid analytical methods in conjunction with olfactometry techniques and traditional sensory taste paneling approaches have greatly improved the understanding of flavour-impact chemicals in cheese and has been used to discriminate between different varieties of cheese.
References

Introduction

In modern times packaging has been identified as an integral part of processing in the dairy and food industry. Package is the gateway to know a product and is brand ambassador of a product. It is the science, art and technology of protecting products from the adverse effects of the environment, and is a media for safe delivery of the contents from the centre of production to the point of consumption. It also serves as a vital link in the long line of production, storage, transport, distribution and marketing. The Importance of foods being properly packaged is seldom noticed in everyday life. The investments made in the manufacture of an excellent product can be a complete failure if the packaging is not appropriate.

From the technical point of view the major advances in cheese making of recent years have been in the mechanization of manufacture and in packaging. There are two distinct requirements for the protection of cheese. From making up to retail distribution the whole cheese must be protected in a transportable form, and for retail distribution and sale, the cut cheese must be protected. If cheese are not protected at all stages they dry out, grow mould, are attacked by mites etc., may lose their shape, and become cracked and broken.

Different types of cheese

- Soft cheese
- Hard cheese
- Semi hard cheese
- Ripened cheese
- Unripened cheese
- Processed cheese
- Processed cheese spread

Requirements of appropriate packaging material

Any material used for packaging of cheese should afford general protection, prevent moisture loss, improve appearance, protect against microorganisms, and prevent oxygen transmission. Broadly speaking, there are two main types of packaging requirement, viz. the long term wrap for factory packaging and the short term wrap for retail sale. The selected packaging material should have good gas barrier properties, mechanical strength, integrity of sealing, type of package, fogging, biodegradability and recyclability, and thermal properties.
For long term wrap for factory packaging, waxed cellulose and nylon films and cellulose – polyfilm, cellulose polythene, polyfilm polythene, and polythene polyester laminates have found favour; and for short term wrap for retail sale the latter, polyfilm, cryovac, saran, polyvinyl are suitable.

**New technologies in cheese industries**

**Type of packaging systems**

- Vacuum packaging
- Active packaging
- Modified atmosphere packaging
- Edible coatings

**Flow pack machines**

These machines are available in two variants, i.e. horizontal flow pack machines and vertical flow pack machines. They allow rapid size change operations (2-3 minutes) without the need to replace mechanical parts, molds or other such operations. A single roll of very fine material is sufficient to pack cheese of different sizes. The cheese is also completely insulated from the outside atmosphere, in close fitting airtight wrappers. The cheese varieties which can be packed by these machines are fresh or mature cheese portions, cheese slices, grated and/or diced cheese and mozzarella type cheese.

**Bio-packs**

- Use of oxygen scavengers, and other preservatives, as active, protective agents in a new bio-based packaging material.
- Improve the shelf life after opening the package at home, reducing the growth of moulds and development of rancid taste.
- The shelf-life of cheese becomes triple from the 2-3 months up to 9 months.

**Metal detector**

According to Lock Inspection Systems, occasional metal contamination is a concern throughout the cheese industry, whether metal swarf (fine metallic filings or shavings removed by a cutting tool) from machinery, fractions of broken cutters and blades or fragments of the clips used to seal packages. The units are fully waterproof and modular construction ensures simplified servicing. The detection units are placed towards the end of each packing line, between the filling and the sealing machines, to detect any metal contamination before the packaging is sealed.

**Smart indicators**

- $O_2/CO_2$ indicators – Redox or pH indicators;
- Microbial growth indicators – pH indicators or certain metabolites indicators
New hard cheese packaging

Hard cheese packed in new biopolymers which will give it an extended shelf-life may be on the shelves in the near future. Substituting fossil plastic materials by renewable biopolymers may benefit the environment and at the same time improve the utilisation of agricultural by-products. According to the Centre for Advanced Food Studies in Denmark, the new biopolymers may be based on proteins like casein, on carbohydrates like starch, cellulose, on lipids, and also on polymers from surplus monomers produced in agriculture such as polylactate (PLA); and finally, on bacterial produced polymers from microorganisms grown on waste, like poly 3-hydroxy-butyrate (PHB). Scientists are dealing with these perspectives for the packaging of hard cheeses: they are developing a new proactive packaging material based on PLA, and are incorporating oxygen scavengers and preservatives encapsulated in cyclodextrins to reduce cheese oxidation (development of rancid taste) and surface growth of moulds.

Cast Polypropylene (CPP) films are one of the leading packaging materials used for film extrusion and has in recent years benefited versus cellophane, metals and paper on account of its superior puncture resistance, low sealing threshold and competitive price. Cast Polypropylene films are available either as cast film or bi-axially orientated polypropylene (BOPP) for packaging of cheese.

Modified atmosphere packaging (MAP)

Modified atmosphere packaging (MAP) is defined as "the packaging of a perishable product in an atmosphere which has been modified so that its composition is other than that of air. English territorial cheeses, e.g. Cheddar have traditionally been vacuum packed. Increasingly MAP is being used now with high CO₂ concentration CO₂/N₂ gas mixes. This has the advantage of obtaining a low residual O₂ content and a tight pack due to the CO₂ going into solution. Use of N₂/CO₂ atmospheres has significant potential for extending the shelf life of cottage cheese. This is high moisture, low fat product that is susceptible to a number of spoilage organisms including *Pseudomonas* spp.

Advantages of MAP

- Modified Atmosphere Packaging, or MAP, extends shelf-life, preserves the high quality of foodstuffs, and improves overall cost-effectiveness.
- Increases sales by satisfying growing demands from consumers for naturally preserved food quality resorting to without additives and preservatives.
- Increases shelf-life quality in the distribution chain by days or even weeks, which increases the availability of fresh food to consumers.
- Reduces the return of spoiled foodstuffs.
- Enhances production efficiency and distribution by cutting costs.
- Increases sales volumes by enabling new products to be offered on new markets.
Conclusion

Revolutionary changes are taking place at a very fast speed in packaging of food products. Since the demand for packaged foods is growing at a rapid rate, it is expected that new forms of packaging materials are likely to appear on the market place for packaging of cheese. The shelf life of high moisture and high fat food products is quite limited mainly due to the chemical effect of atmospheric oxygen and growth of spoilage microorganisms. Both these factors, alone or in conjunction with one another, result in changes in colour, flavour, odour and overall deterioration of the product. Product compression is unavoidable and makes vacuum packaging unsuitable for many products. Modified Atmosphere Packaging (MAP), a modern preservation technique, enhances the shelf life of food products including various varieties of cheese.

References

Introduction

Consumers have the right to expect the food they eat to be safe and suitable for consumption. Outbreak of food borne illnesses can damage trade and tourism, and lead to loss of earnings, unemployment and litigation. Spoilage is wasteful, costly and can adversely affect consumer confidence. In order to produce high microbiological quality fermented milks, the raw materials and other ingredients must also be high quality. The microbiological quality of these ingredients can be monitored by subjecting the samples to standard plate counts to appropriate media for assessing the microbial load. All foods have the potential to cause food borne illnesses, and milk and milk products are no exception. Dairy animals may carry human pathogens and presence of such pathogen in milk may increase the risk of food borne illness. Moreover, the milking procedures, subsequent pooling and storage of milk enhances the risks of further contamination from man or the environment or growth of inherent pathogens. Moreover, the composition of many milk products makes them good media for the outgrowth of pathogenic microorganisms. Therefore, implementing the proper hygienic control during production & processing of Fermented Dairy products is essential in ensuring the safety and suitability of these foods for their intended use.

A variety of pathogenic organisms may gain access into milk and milk products from different sources and cause different types of food borne illnesses. Milk and milk products may carry toxic metabolites of different organisms growing in it. Ingestion of such products, contaminated with these metabolites, cause food poisoning. On the other hand the ingestion of viable pathogenic bacteria along with the food products leads to food borne infection. Some time these organisms undergo lyses in the gastrointestinal tract and liberate toxic substances form inside the cells which are detrimental to the health of consumers. Recent development regarding quality and safety management systems such as ISO and Hazard Analysis Critical Control point (HACCP) has reduced such incidence. Since the international market has become quite demanding in terms of quality and food safety and delivery, installation of quality and food safety measures have become essential for long term survival and for entry into competitive global markets. The application of HACCP is compatible with the implementation of ISO 9000. The HACCP approach adds value to ISO 9000 Quality Management System and a combined effect of both would be a safe and wholesome food to consumer.
HACCP is a management system to assess hazards and establish control systems throughout the food chain from primary production to final consumption that focus on preventative measures rather than relying mainly on end-product testing. It enhances food safety besides better utilization of resources and timely response to problems in the system. It is now widely embraced by the food industries and by the government regulatory agencies around the world as a most cost effective means of minimizing the occurrence of identifiable food borne biological, chemical and physical hazards and maximizing product safety. It is a system which targets critical areas of processing, and in doing so reduces the risk of manufacturing and selling unsafe products.

**Microbiological quality of fermented dairy products**

Microbiological examination of fermented dairy products is done to determine the number of contaminating microorganisms to assure that the material will not undergo microbial spoilage and to provide protection from any potentially pathogenic species in the final products. During its anticipated shelf life; these issues are of vital importance to any company. All contaminating microorganism including bacteria, yeast and mold etc. must also be looked for in the product. For the assessment of processing hygiene, it is worthwhile detecting the presence of Coliforms, staphylococci and Enterococci, since these organisms may survive in low acid fermented milk products. As far as pathogens are concerned, yogurt with an acidity of around 10 kg⁻¹ lactic acid is a fairly inhospitable medium, and really troublesome pathogens like *Salmonella spp.* and *Listeria monocytogenes* will be incapable to growth. Coliforms should also be inactivated by the low pH; in addition, some species may be susceptible to bacteriocins released by the starter organisms. But whether *Staphylococcus spp.* and in particular coagulase-positive strains can survive in yogurt is a matter of some dispute.

**Cheese**

The term cheese covers over 1,000 varieties of fermented dairy products with significant variations in their flavours, texture and appearance. The process of converting liquid milk into cheese involves a series of steps that are modified to produce a cheese of the desired characteristics. Starter culture and rennet are added to milk resulting in the production of a cheese curd through a process of coagulation and acidification. The curds are usually cut and with mild (38-43ºC) heating there is separation of the whey, which is drained from the curds. The curds are salted before they are pressed into moulds and then stored under controlled conditions to ripen the cheese.

**Microbial pathogens of major concern in cheeses**

Cheese has been the vehicle in a number of outbreaks of food-borne illness, involving pathogenic micro-organisms such as *S. aureus*, *Bacillus spp.*, *Salmonella*, *L. monocytogenes*, *E. coli*, *Shigella*, *Cl. botulinum* and *Brucella* spp. (ICMSF, 1998). A full
The list of outbreaks resulting from cheese consumption is provided at Table 1. Evidence from outbreak investigations suggests that illness resulting from consumption of cheese is often the result of faulty controls in cheese production; use of contaminated starter cultures or contaminated ingredients; post-pasteurization contamination; or mishandling during transportation and/or distribution. In microbiological surveys conducted overseas and a number of potential pathogens have been detected in cheeses made from pasteurized milk, namely *L. monocytogenes* and *S. aureus*. Additional pathogens have been detected in raw milk cheese (*B. cereus, Brucella* spp., pathogenic *E. coli* and *Y. enterocolitica*). Detections of *Bacillus* spp. and *L. monocytogenes* have occurred in pasteurized milk cheeses.

**Cultured and fermented milk products**

Yoghurt and fermented milk products are prepared by fermentation of milk or milk products using specific micro-organisms that reduce the pH and coagulate milk proteins. Yoghurt is characterised by fermentation with thermoduric *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* with or without other lactic acid producing bacteria. Fermented milk products include yoghurt, cultured buttermilk, cream (sour cream), and acidophilus milk (Surono et al., 2003).

**Microbial pathogens of major concern**

Fermented products are rarely associated with food-borne disease as their pH is too low and the lactic acid concentration too high to permit growth of vegetative pathogens and death of non-growing cells is likely to be rapid (Varnam and Sutherland, 1994). However, consumption of yoghurt containing large numbers of yeasts can lead to digestive disturbances. The limited outbreaks of food-borne illness that have been reported typically have involved *S. aureus, Cl. botulinum* and *E. coli* 0157:H7 (ICMSF, 1998; O'Mahoney et al., 1990; Morgean et al., 1993). Slow growth by the starter culture provides an opportunity for growth of pathogens that contaminate the milk or ingredients, for example, staphylococcal toxin may accumulate in the ingredients where too much sugar inhibited the growth of starters but not the growth of *S. aureus*, resulting in illness (Mocquot et al., 1970). In yoghurt outbreak, under processing of canned hazel-nut puree used to flavor the yoghurt caused growth and toxigenesis of *C. botulinum* spores in the puree. In addition the sugar in the ingredients was replaced by aspartame, leading to an increase in water activity to a level allowing growth of the pathogen (O'Mahony et al., 1990). From a number of microbiological surveys of cultured and fermented milk products identified in the literature, only one reported the positive identification of a pathogen (*Y. enterocolitica* in fermented cow's milk) (Table-2).

**Hygiene indicators**

A total count or SPC is not suitable for measuring the microbiological quality of these products, because a viable bacterial culture has been added to each of them. Thus, the coliform count is the primary microbiological test that is used in
evaluating fermented dairy products. Many times yoghurt develops a yeast & mold problems as opposed to any bacterial related shelf life ending problems. The standard method for examination of Dairy Products recommends the use of sterile spatula to aseptically transfer 11g of sample into the sterile blender. Then, 99ml of warmed (40-45°C) sterile sodium citrate solution is added followed by thorough mixing for 2 min. Then product is plated with 1ml of the blended 1:10 being transferred to a VRBA Plate (or Petrifilm). In case of suspected colonies, the samples were enriched in Brilliant green lactose bile broth (BGLB). The tubes were incubated at 37°C for 48hrs. This growth in these tubes was then inoculated on Hi-chrome E.coli agar. The plates were incubated at 30°C for 4hrs and 44°C for 18hrs. In case of Yeast & mold acidified potato dextrose agar, yeast extract-dextrose-chloramphenicol agar and dichloran – Rose Bengal chloramphenicol (DRBC) agar are used for enumeration in the products. In addition Petrifilm provides a Yeast and mold agar that is used by many dairy laboratories.

**Safety indicators**

The pathogens like *Staphylococcus aureus*, salmonella, Shigella and *Listeria monocytogenes* are analyzed in fermented dairy products like Dahi, shrikand, lassi and others. These pathogens are analyzed by pre - enrichment, selective enrichment for 24-48hrt in selective pre-enrichment broth and selective enrichment broth recommended by BIS. After pre-enrichment and selective enrichment the broth is streaked on selective medium and incubation at 24-48hrs. Then finally the pathogenic bacteria are confirmed for legal standards (Table -3 & 4) by biochemical identification and further confirmation by molecular techniques like PCR and RT PCR methods.

**The sampling plan**

The following terms, as used by the International Commission on Microbiological Specifications for Foods (ICMSF), have been adopted in developing this draft:

n = the number of sample units which must be examined from a lot of food. Most sampling plans specify taking five sample units. However, when the risk has been assessed as relatively high, a greater number of sample units are specified. This is the case for Salmonella in coconut, cereal-based foods for infants and infant formula where 10 sample units should be examined.

c = the maximum allowable number of defective sample units. This is the number of sample units, which may exceed the microbiological limit specified by ‘m’. These are considered marginal results, but are acceptable providing they do not exceed the limit specified by ‘M’.

m = the acceptable microbiological level in a sample unit. Sampling plans in which m=0 and c=0 are equivalent to ‘absent’ or ‘not detected’ reporting for the stated analytical unit size. In most cases this is 25 g (e.g. not detected in 25 g)
M = the level which, when exceeded in one or more samples, would cause the lot to be rejected.

A lot of food does not comply with the standard if the number of defective sampled units is greater than c, or the level of a micro-organism in a food in any one of the sample units exceeds M.

**Conclusions**

The most of the traditional fermented milk products are prepared by conventional methods in the unorganized sector. Such methods bring about contamination of various microorganisms including pathogens. This has resulted in poor microbiological quality with respect to presence of pathogens and spoilage organism. While the world witnesses technological breakthrough and emerging global markets, global standardization and certification systems encapsulate these developments and provide tools to facilitate international transactions of goods and services. It is in this contest that ISO 9000 Quality Management System Standards brought out by ISO and Food Safety Management Systems (HACCP) standards brought out by the CAC have assumed greater importance for achieving the objective of facilitating global trade for safe and wholesome foods.
<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Cases</th>
<th>Product</th>
<th>Causative Agent</th>
<th>Cause</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>France</td>
<td>45</td>
<td>Brie cheese</td>
<td>Salmonellosis serotype infantis</td>
<td>Milk and factory workers contaminated with Salmonella serotype infantis</td>
<td>(Simon et al., 2002)</td>
</tr>
<tr>
<td>1999</td>
<td>Canada</td>
<td>700</td>
<td>Cheese</td>
<td>S. Enteritidis</td>
<td>Lunch pack products</td>
<td>(CCDR, 1999)</td>
</tr>
<tr>
<td>1996</td>
<td>USA</td>
<td>8 (1)</td>
<td>Cheese Sauce</td>
<td>Clostridium botulinum</td>
<td>A commercial, canned cheese caused a botulism outbreak</td>
<td>(Townes et al., 1996)</td>
</tr>
<tr>
<td>1996</td>
<td>Italy</td>
<td>8</td>
<td>Mascopone cheese</td>
<td>Clostridium botulinum type A</td>
<td>Beak in cold-chain at retail likely caused germination of C. botulinum spores contaminating the products</td>
<td>(Aureli et al., 2000)</td>
</tr>
<tr>
<td>1996</td>
<td>UK</td>
<td>84</td>
<td>Cheddar</td>
<td>S. Goldcoast</td>
<td></td>
<td>(Health Protection Agency 1997)</td>
</tr>
<tr>
<td>1995</td>
<td>Switzerland</td>
<td>57 (16)</td>
<td>Soft cheese</td>
<td>Listeriosis</td>
<td>Consumption of a soft cheese</td>
<td>(Bula et al., 1995)</td>
</tr>
<tr>
<td>1995</td>
<td>USA</td>
<td>9</td>
<td>Cheese</td>
<td>Clostridium perfringens</td>
<td>Consumed in restaurant</td>
<td>(CDC 2002)</td>
</tr>
<tr>
<td>1994</td>
<td>USA</td>
<td>5</td>
<td>Goats cheese</td>
<td>Salmonella enteridis</td>
<td>Consumed in a private home</td>
<td>(CDC 2002)</td>
</tr>
<tr>
<td>1993</td>
<td>USA</td>
<td>12</td>
<td>Cheese slices</td>
<td>Unknown</td>
<td>Consumed at a picnic</td>
<td>(CDC 2002)</td>
</tr>
<tr>
<td>1991</td>
<td>USA</td>
<td>25</td>
<td>Shredded cheese</td>
<td>Unknown</td>
<td>Consumed in a restaurant</td>
<td>(CDC 2002)</td>
</tr>
<tr>
<td>1990</td>
<td>USA</td>
<td>15</td>
<td>Cheese</td>
<td>Hepatitis A</td>
<td>Consumed in a private home</td>
<td>(CDC 2002)</td>
</tr>
<tr>
<td>1990</td>
<td>USA</td>
<td>23</td>
<td>Cheese sauce</td>
<td>S. Braenderup</td>
<td>Consumed in restaurant</td>
<td>(CDC 2002)</td>
</tr>
<tr>
<td>1990</td>
<td>USA</td>
<td>12</td>
<td>Processed Cheese</td>
<td>S. Enteritidis</td>
<td>Consumed in hospital</td>
<td>(CDC 2002)</td>
</tr>
<tr>
<td>Year</td>
<td>Country</td>
<td>Cases</td>
<td>Product</td>
<td>Causative Agent</td>
<td>Cause</td>
<td>Reference</td>
</tr>
<tr>
<td>------</td>
<td>---------</td>
<td>-------</td>
<td>---------</td>
<td>----------------</td>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td>1989</td>
<td>USA</td>
<td>167</td>
<td>Contaminated cheese</td>
<td>S. Javiana and S. Oranienburg</td>
<td>Mozzarella cheese manufactured at a single cheese plant</td>
<td>(Hedberg et al., 1992)</td>
</tr>
<tr>
<td>1983</td>
<td>USA</td>
<td>45</td>
<td>French Brie cheese</td>
<td>E. coli O27:H20</td>
<td>Three clusters of gastrointestinal illness, after office parties</td>
<td>(MacDonald et al., 1985)</td>
</tr>
<tr>
<td>1982</td>
<td>Canada</td>
<td>?</td>
<td>Cheddar Cheese</td>
<td>S. Typhimurium</td>
<td></td>
<td>(D’Aoust, 1985)</td>
</tr>
<tr>
<td>1976</td>
<td>USA</td>
<td>28,000 to 36,000</td>
<td>Cheddar cheese</td>
<td>S. Heidelberg</td>
<td>Consumption of cheddar cheese from a single shipment of a single manufacturer</td>
<td>(Fontaine et al., 1980)</td>
</tr>
</tbody>
</table>

**Table 2: Outbreaks of illness associated with Yogurt and Fermented Milk**

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Cases</th>
<th>Product</th>
<th>Causative Agent</th>
<th>Cause</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>UK</td>
<td>16</td>
<td>Yoghurt</td>
<td>E. coli O 157</td>
<td>Consumption of a locally produced live yoghurt</td>
<td>(Morgan et al., 1993)</td>
</tr>
<tr>
<td>1989</td>
<td>UK</td>
<td>27(1)</td>
<td>Hazelnut flavoured yoghurt</td>
<td>Clostridium botulinum type B toxin</td>
<td>Can of hazelnut conserve, opened and unopened cartons of hazelnut yoghurt</td>
<td>(O’Mahony et al., 1990)</td>
</tr>
</tbody>
</table>
Table-3: Draft PFA Microbiological standards for Yoghurt, Dahi, Chakka & Srikhand

<table>
<thead>
<tr>
<th>Microbiological requirements</th>
<th>Sampling plan</th>
<th>Draft PFA standards</th>
<th>Standard reference of testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform Count</td>
<td>5</td>
<td>2</td>
<td>10/g 50/g</td>
</tr>
<tr>
<td>E. coli</td>
<td>5</td>
<td>0</td>
<td>Absent /g</td>
</tr>
<tr>
<td>Salmonella</td>
<td>5</td>
<td>0</td>
<td>Absent /25 g</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>5</td>
<td>2</td>
<td>50/g 100/g</td>
</tr>
<tr>
<td>YMC</td>
<td>5</td>
<td>2</td>
<td>50/g 100/g</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>5</td>
<td>0</td>
<td>Absent/g</td>
</tr>
</tbody>
</table>

Table-4: BIS microbiological standards for fermented dairy products

<table>
<thead>
<tr>
<th>Microbiological requirements</th>
<th>Dahi Shrikan d Chakka Yoghurt</th>
<th>Standard reference of testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Lactic acid bacteria</td>
<td>-</td>
<td>- 10,00,000/g m</td>
</tr>
<tr>
<td>Coliform Count</td>
<td>10/g 10/g 10/g -</td>
<td>Absent/g</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
<td>Absent/g</td>
</tr>
<tr>
<td>Salmonella</td>
<td>-</td>
<td>Absent/25g</td>
</tr>
<tr>
<td>Shigella</td>
<td>-</td>
<td>Absent/25g</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>-</td>
<td>Absent/g</td>
</tr>
<tr>
<td>YMC</td>
<td>100/g 50/g 20/g -</td>
<td>IS : 5403 - 1999</td>
</tr>
</tbody>
</table>

References


• International Commission on Microbiological Specifications for Foods (1986) Micro-organisms in Foods, Sampling for Microbiological Analysis; Principles and Specific Applications


• Microbiological reference criteria for food (1995) developed by Australia new Zealand food authority.


• Principles for the Establishment and Application of Microbiological Criteria for Foods, CAC/GL 21-1997


• SOHRAB. ISO 9000 and HACCP in food industry. Indian Institute of Packaging, Mumbai.


Production of whey-based beverages started in 1970’s and until today a wide range of different whey beverages has been developed. It is estimated that about 3-million tons/annum of whey which is generated in India and contains about 2-lack tons of precious milk constituents. Whey a by-product in the manufacturing of dairy products mainly cheese, paneer and shows great potential for the development of dairy products due its nutritional value since it is not only a source of the most biological valuable proteins but also rich in proteins, but also rich in minerals and vitamins mainly riboflavin. Whey constitute about 80-90% of the volume of milk used for conversion into chhana, paneer, cheese and casein. When it is drained off, there is a great loss of nutrients, creating a serious problem of environmental pollution. It has been noted that while in developed countries about 95% of the total whey is used in food production, in Brazil only 50% is utilized. In India, there has been a substantial increase in the production of paneer, resulting in an increased accessibility of whey. Biological waste water treatment technologies can assist in the safe disposal of whey or whey permeates within the federal environment specifications, but only at substantial cost. Consequently, exhaustive research work has been carried out to explore the ways of whey utilization in various forms such as whey protein concentrates (WPC), condensed whey, sweetened whey, whey-based beverages, alcohol production, whey based media and lactic acid production. The addition of cheese whey, probiotic bacteria, and prebiotics to a lactic beverage could result in a functional food, serving as a new alternative for the dairy industry and for consumers interested in a healthy, nutritious diet; it also has new sensorial characteristics.

Functional dairy beverages: The products of this group demonstrate health benefits beyond their basic nutritional value. These products are enriched with functional food components originating from dairy and nondairy sources. Probiotics are the main bioactive components of fermented functional dairy foods and numerous economic indicators show that probiotic-enriched products are still in the forefront of innovation in the functional food sector (Champagne 2009). The dairy-based beverages market is still a niche market compared with the sales of yogurt and plain milk, and dairy beverages containing probiotics and/or prebiotics dominate the functional dairy beverages market. Apart from milk-based beverages, whey- or soya-based functional beverages are also gaining popularity. Fermented beverages constitute an important part of the human diet because fermentation is one of the cheapest ways of preserving the food, improving its nutritional value, and
enhancing its sensory properties. Functional dairy beverages can be categorized into two basic groups:

- **a) Fortified dairy beverages** (including probiotics, prebiotics/fibres, polyphenols, peptides, sterol/stanols, minerals, vitamins and fish oil)

- **b) Whey-based beverages** (both fruit juice-type and dairy-type)

**Probiotic dairy beverages**

Probiotics are defined as ‘live microorganisms which when consumed in adequate numbers confer a health benefit on the host’ (FAO/WHO, 2001), with ongoing controversy as to whether cultures must be viable for efficacy in all cases. Prebiotics are nondigestible food ingredients that beneficially affect the host by stimulating growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health. Microorganisms might also indirectly impart health-promoting characteristics in food through the production of bioactive metabolites (referred to as biogenics) during fermentation. Consumption of probiotic bacteria via food products is an ideal way to reestablish the balance of intestinal microbiota. These include alleviation of lactose intolerance symptoms, lowering cholesterol, curing antibiotic-associated diarrhoea, prevention of intestinal tract infections, prevention of colon cancer, control of rotavirus, prevention of ulcers related to *Helicobacter pylori*, improvement of immune system, irritable bowel syndrome and antihypertensive effects. In order to produce therapeutic benefits, a suggested range for the minimum level for probiotic bacteria in probiotic milk is from $10^6$ to $10^7$ colony-forming units (cfu)/mL (IDF 1992). In order to improve the viability of probiotic bacteria in fermented milks various substances have been added to milk. These substances include fructo-oligosaccharides (FOS), caseinomacropeptides (CMP), whey protein concentrate (WPC), tryptone, yeast extracts, certain amino acids, nucleotide precursors and an iron sources.

**Whey based fermented beverages**

Whey is a by-product of cheese, paneer, chhana and coagulated dairy products. It is an important source of lactose, calcium, milk proteins and soluble vitamins, which make this product to be considered as a functional food and a source of valuable nutrients. In India, there has been a substantial increase in the production of paneer, resulting in an increased accessibility of whey. India’s annual production is estimated at 1, 50,000 tones of paneer and concerning 2 million tones of whey, containing about 130000 tones of valuable milk nutrients are produced per annum. Growing environmentalist concern have made dumping expensive as it is containing high B.O.D. and C.O.D. while the development of technology has opened up new and cost effective ways of utilizing the whey constituents which has helped to find a wide range of new applications and the development of dairy industry.
Whey products have certain essential amino acids, good digestibility, and protein efficiency index higher than 3.0. Vitamins such as thiamin, riboflavin, pantothenic acid, vitamin B6 and B12 are also present. So far the whey is considered to be a waste product in the dairy industry but process has been developed to produce a healthy drink from this waste material. This beverage unlike the other carbonated beverages which are of little usefulness has following advantages:

- It has a good nutritional value
- It has therapeutic values namely: (a) protection against gastro-intestinal disorders and (b) bio-availability of vitamins.
- It has prolonged shelf life under refrigeration condition (4 to 7°C).
- It is much cheaper in cost compared to the other known and available beverages or, carbonated drinks.

The microorganisms used in these beverages include certain selected species of probiotic and non-probiotic lactic acid bacteria (single or mixed) and yeast cultures.

**Whey-based beverages**

Gandhi (1989) developed and patented process of fermented whey drinks named as ‘Acidowhey’. This is a refreshing and palatable beverage from paneer whey. In the process, pasteurisation of fresh paneer whey at 72°C for 15 mins was carried out followed by cooling to 37°C and then addition of a culture of lactobacillus acidophilus @ 1 % and fermented at 37°C for 24 hrs. Sugar and citrus flavour are then added to make the product palatable and stored in refrigerated conditions. Final product had a typical acidity between 0.8 - 0.9 % (LA). The standard product was found to be free from yeast and mold and coliforms. The shelf life of the product was reported to be 2-3 weeks at refrigerated conditions.

A cultured whey drink was prepared from raw milk. Whey was heated at 121°C for 20 min. and cooled at 40°C. The *Bifidobacterium bifidum* starter culture and flavouring material were added and incubated anaerobically at 37°C for about 12 hrs and the *B. bifidum* count in the final product (pH: 4-5) was 10⁶ cfu/ml, coliform were less than 40/ml and pathogens were absent. The product remained fit for consumption for 7 days at refrigerated condition. "Wheyvit", an orange or pineapple flavoured beverage from whey was developed (Bambha et al., 1972). A culture of *Saccharomyces cerevisiae* was used to ferment deproteinised whey. Fresh cheese whey was separated to .05 % fat, deproteinized by heating at 80°C for 10 min. and then cooled to room temperature. 50 % sugar solution was added @ 22-23% of whey, followed by the addition of 10 % citric acid solution @ 2-2.2 % and color and flavour to the desirable intensity. *S. cerevisiae* culture was added @ 1% thereafter, incubation at 22°C for 14- 16 hrs. Final product had a pleasant flavour and contained 0.5-0.7 % alcohol. The product was found good and fit for human consumption for a period of 7-8 days when stored at 5°C.
Rivella, a sparkling, crystal clear infusion of alpine herbs, first appeared in Switzerland in 1952. Rivella was prepared by fermenting deproteinised whey with lactic acid bacteria, filtering, condensing to a 7:1 concentrate, adding sugar and flavouring, refrigering, diluting and carbonating, and after which the product was bottled and pasteurised. The finished beverage cinatined 9.7 % TS, 0.125% total nitrogen and the pH were about 3.7. Rivella is the most successful soft drink in Switzerland that is a head of Coca-Cola.

In the present scenario of consumption of fermented whey drinks such as Molke in West Germany, Rivella in Switzerland etc. and these products are showing increasing trends in most of the countries around the world. Keeping in view increased demand of soft drinks and juices these days in India, there is a tremendous scope and need to exploit commercial production of these fermented whey beverages since it is the best proposition to convert largest by-product (whey) of dairy industries into value added product by simple and indigenous processes. Whey based fermented beverages are available in the market as lactic fermented acido whey soft drink, alcoholic fermented wine and beer, low alcoholic whey beverages fortification with grape juice, probiotic containing whey based drinks. These whey based drinks are nutritious, thirst quenching and therapeutic in nature.

One of the better options for beverages with acceptable sensory properties is production of fermented whey beverages. Special attention is being paid for development of fermented whey beverages with probiotic bacteria. Since the beneficiary effects of probiotic strains on human health like lowering cholesterol level in blood, improving lactose metabolism, lowering blood pressure, anticancreogenic properties and immune system stimulation are known for a long period of time (Shah, 2007). WPC 35%, w/w protein, powder was reconstituted with distilled water to 10% and the pH was adjusted to 8.0 with 2mol/1 Na. The reconstituted WPC35 was heat treated at 116 °C for 20 min, stored at 4 °C until use (no longer than one week) and used as fermentation medium.

WPC35 was allowed to ferment for 12 h, cooled down in ice and diluted 1:3 with peach juice (ZUCO, Corandes S.A., Argentina), previously dissolved in sterile water or calcium lactate 2% (w/v). Calcium lactate was added as acidity regulator following the indications of the Codex Alimentarius (CODEX STAN, 192-1995). The resulting beverages were distributed in sterile plastic bottles in triplicates and stored at 10 °C for 28 days. Viable cell count, pH, sugar and lactic acid concentrations, proteolytic activity, free amino acid content and whey protein degradation were determined after 0, 7, 14, 21 and 28 days of storage (Mendoza et al., 2010).

**Benefits of whey based fermented drinks:**

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

References

Introduction

Soybeans have been part of food history in Asia for several millennia but did not reach the Americas and Europe until the eighteenth century. In the twentieth century, there was a tremendous increase in the cultivation of soybeans in the United States and more recently in South America. Soy foods have entered the U.S. food supply in ever-increasing amounts both in the form of traditional products (soy milk, tofu) and in more subtle ways in dairy and bread/cake products. Soymilk is lactose free and a good source of essential fatty acids. It contains no cholesterol and little or no saturated fat. Soymilk can be a good source of high quality protein, B vitamins, potassium, iron, dietary fiber, and bio-active components, including isoflavones. Many soymilks are fortified with calcium, vitamins A and D, riboflavin, zinc, and vitamin B12. Important bio-active components, found naturally in soybeans are being studied in relation to relieving menopausal symptoms, such as hot flashes, maintaining healthy bones, and preventing prostate, breast cancers, and colorectal cancer. The content and profile of bio-active components varies from product to product, depending upon how much soy protein is in the food and how the soy protein is processed. Soymilk is a healthy, high-quality protein source that contains all essential amino acids needed for growth. In general, soy protein products are equal in quality to animal products.

In addition to the excellent nutritional value of soy protein, scientists have found that consumption of soy protein can contribute to reducing the risk of heart disease. The FDA has approved a health claim stating that “25 grams of soy protein in a daily diet low in saturated fat and cholesterol can help reduce total and LDL cholesterol that is moderately high to high.” Soymilk is curdled to prepare tofu, which can be pressed to remove water. Tofu can be fried or added to numerous other dishes. In the United States, soybeans are grown mostly as a source of edible oil using a hexane extraction approach. As in Asia, these soymilks are converted to tofu. However, this is typically done aseptically producing shelf-stable products. In a recent development, soy products are being made where microorganisms that hydrolyze the isoflavones are added to soy protein preparations.

Soy based fermented foods:

Soy based probiotic yoghurt

Soy fortified yoghurt is a nutraceuticals food. Yoghurts with 5% added soy protein concentrate qualify for the FDA-approved soy health claim of “cholesterol reducing”
and also contain sufficient fiber to provide 1 g of dietary fiber per serving. Soy yoghurt is a fermented product made with a mixed starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* (Lee and Morr, 1990)

In dairy and soy applications, probiotic organisms are delivered with different fermented dairy and soy products, most notably yoghurt and soy yoghurt. Incorporation of probiotic organisms such as *Lactobacillus acidophilus*, *Bifidobacterium* sp., and *L. casei* in fermented products provides a potential to improve the quality of the product and the health status of consumers. Dairy and soy foods may serve as the ideal system for delivery of probiotic bacteria to the human gastrointestinal tract (GIT) due to provision of a favorable environment that promotes the growth and enhances the viability of these microorganisms. During the bacterial fermentation, major constituents including lactose and milk proteins, soy proteins, raffinose, stachyose and other soy carbohydrates are utilized for the bacterial growth, which results in the conversion of fermentable materials into a range of products such as lactic acid, acetic acid, peptides, amino acids and different vitamins. In addition to exceptional nutritional attributes, milk and soy derived products such as fermented soy yoghurt or soy contain components that possess a range of different bioactive compounds. Compared to casein, soy protein showed a greater antioxidative ability in preventing lipid oxidation. Isoflavones have been found to increase the activities of some antioxidative enzymes in the liver. Some of these bioactive compounds are considered functional, thus making dairy and soy products important part of functional foods and nutraceuticals (Cherry *et al.*, 2002)

**Soy shrikhand**

Shrikhand was prepared as per the method described by De (1980) from different blends of soy milk and dairy milk in different ratio. The blends were homogenized in 2 stages using baby homogenizer (Gaulin, APV, USA). In the 1st stage, pressuer was maintained at 150kg/cm² and in the 2nd stage pressure was 200 kg/cm². Finally milk was heated at 72°C for 20 min and cooled to room temperature (30-37°C). Dahi @1% was then inoculated to all the blends and incubated at 37°C for 6 h (Trenna and Stanley, 2005). The solid material called Chakka was prepared from the dahi and 60% sugar was added. The contents were througly mixed and then screened through fine muslin cloth. The consumer acceptance of shrikhand decreased with increase in soy milk supplementation. The highest acceptable soy milk supplementation was 60%.

**Soymilk kefir**

Soy milk is prepared from soy beans (Mital *et al.*, 1974). Then kefir grains (*Lb. brevis*, *Lb. helveticus*, *Lb. kefir*, *Leuconostoc mesenteroides*, *Kluyveromyces lactis*, *K. marxianus*, *Pichia fermentans*) are added @5% to the milk. Kefir grains are small, gelatinous, yellowish, and irregularly shaped masses resembling individual
miniature florets of a head of cauliflower. Yeast and lactic acid bacteria coexist as symbiotic relationship for an acid-alcohol fermentation (Liu and Lin, 2000). The microorganisms constituting the kefir grains produce lactic acid, antibiotics, and bacteriocide which inhibit the development of degrading and pathogenic microorganisms in kefir milk. Kefiran is reported to possess antitumor activity (Shiomi et al., 1982).

**Soy beverages**

Soybeans (250g) were initially soaked in 1 L of water for 16 hr at 25°C. Subsequently, 190g of soaked beans in 500 ml of water were ground with a commercial blender unit for 3 min, filtered on whatman #4 membranes and boiled for 5 min. This milk contains 4.5% protein and 2.3% fat (Champagne et al., 2009).

Tofu or bean curd is a food made by coagulating soy milk and then pressing the resulting curds into soft white blocks. Tofu is made by coagulating soy milk and pressing the resulting curds. Although pre-made soy milk may be used, most tofu producers begin by making their own soy milk, which is produced by soaking, grinding, boiling and straining dried (or, less commonly, fresh) soybeans (William and Akiko, 2006). Coagulation of the protein and oil (emulsion) suspended in the boiled soy milk is the most important step in the production of tofu. This process is accomplished with the aid of coagulants. Two types of coagulants (salts-calcium sulfate and acids-Glucono delta-lactone) are used commercially. The third type of coagulant, enzymes, is not yet used commercially but shows potential for producing both firm and "silken" tofu. Tofu and its production technique were introduced into Korea and then Japan. Tofu contains a low amount of calories, relatively large amount of iron, and little fat. Depending on the coagulant used in manufacturing, the tofu may also be high in calcium and/or magnesium. 100 grams of firm tofu contains 15.78 grams of soy protein. The FDA granted this health claim for soy: "25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease" (Guo and Ono, 2005).

**Cheonggukjang:** is a fermented soybean paste used in Korean cuisine. It contains whole as well as ground soybeans. It can be made in 2 to 3 days through fermentation of boiled soybeans, adding Bacillus subtilis, which is usually contained in the air or in the dried rice plants, at 40 °C without adding salt, compared with the much longer fermentation period required for doenjang, another, less pungent variety of Korean soybean paste. Like many forms of doenjang, cheonggukjang is paste-like in texture, but also includes some whole, uncrushed soybeans. Cheonggukjang may also be made by fermenting boiled soybeans in a warm place, pounding a portion of them, and adding salt and red chili powder. Cheonggukjang is generally considered to be a healthy food (particularly in the winter), as it is rich in vitamins and other nutrients, though its very strong odor is not universally enjoyed. Some people have commented that this soup gives off the aroma of wet socks.
**Doenjang:** is a traditional Korean fermented soybean paste. Its name literally means "thick paste" in Korean (Ja, 2008). To produce *doenjang*, dried soybeans are boiled and stone-ground into coarse bits. This paste is then formed into blocks, which are called meju. The blocks are then exposed to sunlight or warmth. When they are exposed to the sun or warmth, dried rice plants are attached to the surface of the soybean blocks. Dried rice plants are readily available in Korea and are a rich source of bacteria (*Bacillus subtilis*). The fermentation process begins at this stage. The *Bacillus subtilis* bacteria reproduce, consuming soybean protein and water in the meju. The unique smell of the meju is mainly the ammonia which is produced by the bacteria. One to three months later, depending on the block size, the meju are put into large opaque pottery jars with brine and left to further ferment, during which time various beneficial bacteria transform the mixture into a further vitamin-enriched substance (similar to the way milk ferments to become yogurt). Liquids and solids are separated after the fermentation process, and the liquid becomes Korean soy sauce (Park, 2007). The solid, which is *doenjang*, is very salty and quite thick, often containing (unlike most miso) some whole, uncrushed soybeans. *Doenjang* is rich in flavonoids and beneficial vitamins, minerals, and hormones which are sometimes claimed to possess anti-carcinogenic properties. In Korean traditional meals, the menu has concentrated on vegetables and rice but *doenjang*, which is made of soybeans, has a great deal of lysine, an essential amino acid that rice lacks. There are linoleic acid of 53% and linolenic acid of 8% which have an important role in normal growth of blood vessels and prevention of blood-vessel-related illness.

**Reference**


Introduction

Cheese is a nutritious, versatile dairy food. To fulfill the consumer demand, to meet the specific consumer requirements and convenience of use wide variety of cheeses are coming into market. Cheese contains a high concentration of essential nutrients relative to its energy level. The nutrient content of cheese is greatly influenced by the type of milk used (species, stage of lactation, full-fat, low-fat, skim), the method of manufacture and, to a lesser extent, the degree of ripening. The water-insoluble nutrients of milk (coagulated casein, colloidal minerals, fat, fat-soluble vitamins) are retained in the cheese curd whereas the water-soluble milk constituents (whey proteins, lactose, water-soluble vitamins and minerals) partition into the whey.

Milk and dairy products, including cheese, contain components which may increase the risk of certain chronic diseases but reduce the risk of others (Norat and Riboli, 2003). Cholesterol and saturated fat are potential risk factors for atherosclerosis. A recent paper (Moss and Freed, 2003) has suggested that non-fat constituents of milk, particularly the calcium magnesium ratio, lactose and milk fat globule membrane antigens, have specific coronary atherogenic effects. However, other components may reduce risks, e.g., conjugated linoleic acid (CLA) which may have antioxidant and anticancer properties, calcium which may protect against hypertension and osteoporosis, and folic acid, vitamin B6 and vitamin B12 which may exert beneficial effects on plasma homocysteine levels (an independent risk factor for atherosclerosis).

The epidemiological evidence for an association between dairy products, including cheese, and colorectal cancer has been reviewed by Norat and Riboli (2003); no significant association between cheese consumption and colorectal cancer was noted. Epidemiological studies which attempt to investigate the effect of a specific food item (e.g., cheese) on disease risk are fraught with difficulty in interpretation as it is more likely that it is the overall dietary profile, made up of balance of wide variety of different foods, which may influence risk of chronic disease.
Table 1 Composition of selected cheeses, per 100 g (Holland et al., 1989)

<table>
<thead>
<tr>
<th>Cheese type</th>
<th>Water (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Carbohydrate (g)</th>
<th>Cholesterol (mg)</th>
<th>Energy (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brie</td>
<td>48.6</td>
<td>19.3</td>
<td>26.9</td>
<td>Tr</td>
<td>100</td>
<td>319</td>
</tr>
<tr>
<td>Camembert</td>
<td>50.7</td>
<td>20.9</td>
<td>23.7</td>
<td>Tr</td>
<td>75</td>
<td>297</td>
</tr>
<tr>
<td>Cheddar (normal)</td>
<td>36</td>
<td>25.5</td>
<td>34.4</td>
<td>0.1</td>
<td>100</td>
<td>412</td>
</tr>
<tr>
<td>Cheddar (reduced-fat)</td>
<td>47.1</td>
<td>31.5</td>
<td>15</td>
<td>Tr</td>
<td>43</td>
<td>261</td>
</tr>
<tr>
<td>Cottage cheese</td>
<td>79.1</td>
<td>13.8</td>
<td>3.9</td>
<td>2.1</td>
<td>13</td>
<td>98</td>
</tr>
<tr>
<td>Cream cheese</td>
<td>45.5</td>
<td>3.1</td>
<td>47.4</td>
<td>Tr</td>
<td>95</td>
<td>439</td>
</tr>
<tr>
<td>Danish blue</td>
<td>45.3</td>
<td>20.1</td>
<td>29.6</td>
<td>Tr</td>
<td>75</td>
<td>347</td>
</tr>
<tr>
<td>Edam</td>
<td>43.8</td>
<td>26</td>
<td>25.4</td>
<td>Tr</td>
<td>80</td>
<td>333</td>
</tr>
<tr>
<td>Emmental</td>
<td>35.7</td>
<td>28.7</td>
<td>29.7</td>
<td>Tr</td>
<td>90</td>
<td>382</td>
</tr>
<tr>
<td>Feta</td>
<td>56.5</td>
<td>15.6</td>
<td>20.2</td>
<td>1.5</td>
<td>70</td>
<td>250</td>
</tr>
<tr>
<td>Fromage frais</td>
<td>77.9</td>
<td>6.8</td>
<td>7.1</td>
<td>5.7</td>
<td>25</td>
<td>113</td>
</tr>
<tr>
<td>Gouda</td>
<td>40.1</td>
<td>24</td>
<td>31.0</td>
<td>Tr</td>
<td>100</td>
<td>375</td>
</tr>
<tr>
<td>Mozzarella</td>
<td>49.8</td>
<td>25.1</td>
<td>21.0</td>
<td>Tr</td>
<td>65</td>
<td>289</td>
</tr>
<tr>
<td>Parmesan</td>
<td>18.4</td>
<td>39.4</td>
<td>32.7</td>
<td>Tr</td>
<td>100</td>
<td>452</td>
</tr>
<tr>
<td>Processed cheese*</td>
<td>45.7</td>
<td>20.8</td>
<td>27</td>
<td>0.9</td>
<td>85</td>
<td>330</td>
</tr>
<tr>
<td>Ricotta</td>
<td>72.1</td>
<td>9.4</td>
<td>11</td>
<td>2</td>
<td>50</td>
<td>144</td>
</tr>
<tr>
<td>Roquefort</td>
<td>41.3</td>
<td>19.7</td>
<td>32.9</td>
<td>Tr</td>
<td>90</td>
<td>375</td>
</tr>
<tr>
<td>Stilton</td>
<td>38.6</td>
<td>22.7</td>
<td>35.5</td>
<td>0.1</td>
<td>105</td>
<td>411</td>
</tr>
</tbody>
</table>

**Proteins**

The concentration of protein in cheese varies from approximately 3% to 40%, depending on the variety (Table 1). During traditional cheese manufacture, most of the whey proteins pass into the whey. Whey proteins represent only 2-3% of the total protein in cheese. Cheese protein is predominantly casein, as the vast majority of the whey proteins are lost in the whey. As casein is slightly deficient in sulfur-containing amino acids, the biological value of cheese protein is slightly less than that of total milk protein. If the essential amino acid index of total milk protein is assigned a value of 100, the corresponding value for cheese protein varies from 91 to 97, depending on the variety. If whey proteins are incorporated into cheese, such as by use of ultrafiltration, the biological value of cheese protein is similar to that of total milk protein.

Cheese ripening typically involves the progressive breakdown of casein by indigenous milk enzymes, rennet, and bacterial enzymes into water-soluble and water-insoluble peptides and amino acids. This process is essential for the development of flavor and also increases the digestibility of cheese protein to almost 100%.

Milk proteins are key source of a range of bioactive peptides. These peptides may be released from their parent protein by proteolysis in products such as cheese. The
production of bioactive peptides is influenced by the starter culture and ripening conditions. An important class of bioactive peptides are peptides that inhibit the activity of angiotensin 1-converting enzyme (ACE), inhibition of which mainly gives rise to antihypertensive effects but may also modulate immuno-defense and nervous system activity. Angiotensin I-converting enzyme-inhibitory peptides have been reported in several ripened cheeses. It appears that the bioactive peptides liberated by starter proteolytic enzymes during cheese ripening can be degraded further to inactive fragments, as the ripening progresses. For example, an antihypertensive peptide derived from αs1-casein was observed in 6-month-old Parmesan cheese but was not detected in 15-month-old cheese. Anticancer effects have been reported for peptides derived from a slurry of cheese made using Lc. lactis subsp. lactis as a starter culture (Kim et al., 1995). Bioactive peptides have potential as ingredients in functional foods and pharmaceuticals.

**Carbohydrate**

Most of the lactose, the principal carbohydrate in milk, is lost in whey during cheese manufacture and hence most cheeses contain only trace amounts of carbohydrate (Table 1). Furthermore, the residual lactose in cheese curd is usually fermented to lactic acid by the starter bacteria. Thus, cheeses can be consumed without ill-effects by lactose-intolerant individuals who are deficient in the intestinal enzyme, P-galactosidase.

**Fat and cholesterol**

Fat plays several important functions in cheese: it affects cheese firmness, adhesiveness, mouth-feel, and flavor. It also contributes significantly to the nutritional properties of cheese, as most cheeses contain significant amounts of fat. For example, a 50 g serving of Cheddar cheese provides 17 g fat, in which approximately 66% of the fatty acids are saturated, 30% are monounsaturated, and 4% are polyunsaturated. Thus, cheese contributes a significant amount of both saturated fat and total fat to the diet.

The cholesterol content of cheese varies from approximately 10 to 100 mg/100 g, depending on the variety. In recent years, there has been considerable research interest in the role of ingested cholesterol oxidation products (COPs) in the etiology of chronic diseases. However, under normal conditions of manufacture, ripening, and storage, negligible amounts of COPs are formed in cheese. Conjugated linoleic acid (CLA) is a potentially beneficial component of milk products, including cheese. Conjugated linoleic acid is a mixture of positional and geometric isomers of linoleic acid that contain conjugated unsaturated double bonds. The principal isomer is cis-9, trans-1 1-octadecadienoic acid which accounts for more than 82% of total CLA in dairy products. Conjugated linoleic acid has been reported to have antioxidant and anticarcinogenic properties in vitro and in animal models (Ip et al., 1991).
On average, the concentration of CLA in milk and dairy products ranges from 0.2 to 1.6 g/100 g fat. Feta and hard cheeses contain 1.9g (average of 0.8) CLA/100 g fat.

**Vitamins**

The concentration of fat-soluble vitamins in cheese is influenced by the same factors that affect its fat content. Most of the fat-soluble vitamins in milk are retained in the cheese fat. The concentration of water-soluble vitamins in cheese is generally lower than in milk due to losses in the whey. The loss of some of the B vitamins is offset, to a certain extent, by microbial synthesis during cheese ripening. In particular, propionic acid bacteria synthesize significant levels of vitamin B12 in hard cheeses such as Emmental (Renner, 1987). In general, most cheeses are good sources of vitamin A, riboflavin, vitamin B12 and, to a lesser extent, folate. The vitamin content of a range of cheeses is shown in Table 2 (Holland et al., 1989). Cheese contains negligible levels of vitamin C.

**Table 2 Vitamin content of selected cheeses, per 100 g (Holland et al., 1989)**

<table>
<thead>
<tr>
<th>Cheese type</th>
<th>Retinol (µg)</th>
<th>Carotene (µg)</th>
<th>Vitamin D (µg)</th>
<th>Vitamin E (mg)</th>
<th>Thiamine (mg)</th>
<th>Riboflavin (mg)</th>
<th>Niacin (mg)</th>
<th>Vitamin B6 (mg)</th>
<th>Vitamin B12 (µg)</th>
<th>Folate (µg)</th>
<th>Pantothenat (mg)</th>
<th>Biotin (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brie</td>
<td>285</td>
<td>210</td>
<td>0.2</td>
<td>0.84</td>
<td>0.04</td>
<td>0.43</td>
<td>0.43</td>
<td>0.15</td>
<td>1.2</td>
<td>58</td>
<td>0.35</td>
<td>5.6</td>
</tr>
<tr>
<td>Camembert</td>
<td>230</td>
<td>315</td>
<td>0.18</td>
<td>0.65</td>
<td>0.05</td>
<td>0.52</td>
<td>0.96</td>
<td>0.22</td>
<td>1.1</td>
<td>102</td>
<td>0.36</td>
<td>7.6</td>
</tr>
<tr>
<td>Cheddar (normal)</td>
<td>325</td>
<td>225</td>
<td>0.26</td>
<td>0.53</td>
<td>0.03</td>
<td>0.4</td>
<td>0.07</td>
<td>0.1</td>
<td>1.1</td>
<td>33</td>
<td>0.36</td>
<td>3</td>
</tr>
<tr>
<td>Cheddar (reduced fat)</td>
<td>165</td>
<td>100</td>
<td>0.11</td>
<td>0.39</td>
<td>0.03</td>
<td>0.53</td>
<td>0.09</td>
<td>0.13</td>
<td>1.3</td>
<td>56</td>
<td>0.51</td>
<td>3.8</td>
</tr>
<tr>
<td>Cottage cheese</td>
<td>44</td>
<td>10</td>
<td>0.03</td>
<td>0.08</td>
<td>0.03</td>
<td>0.26</td>
<td>0.13</td>
<td>0.08</td>
<td>0.7</td>
<td>27</td>
<td>0.4</td>
<td>3</td>
</tr>
<tr>
<td>Cream cheese</td>
<td>385</td>
<td>220</td>
<td>0.27</td>
<td>1.0</td>
<td>0.03</td>
<td>0.13</td>
<td>0.06</td>
<td>0.04</td>
<td>0.3</td>
<td>11</td>
<td>0.27</td>
<td>1.6</td>
</tr>
<tr>
<td>Danish blue</td>
<td>280</td>
<td>250</td>
<td>0.23</td>
<td>0.76</td>
<td>0.03</td>
<td>0.41</td>
<td>0.48</td>
<td>0.12</td>
<td>1.0</td>
<td>50</td>
<td>0.53</td>
<td>2.7</td>
</tr>
<tr>
<td>Edam</td>
<td>175</td>
<td>150</td>
<td>0.19</td>
<td>0.48</td>
<td>0.03</td>
<td>0.35</td>
<td>0.07</td>
<td>0</td>
<td>2.1</td>
<td>40</td>
<td>0.38</td>
<td>1.8</td>
</tr>
<tr>
<td>Emmental</td>
<td>320</td>
<td>140</td>
<td>N</td>
<td>0.44</td>
<td>0.05</td>
<td>0.35</td>
<td>0.1</td>
<td>0.09</td>
<td>2</td>
<td>20</td>
<td>0.4</td>
<td>3</td>
</tr>
<tr>
<td>Feta</td>
<td>220</td>
<td>33</td>
<td>0.5</td>
<td>0.37</td>
<td>0.04</td>
<td>0.21</td>
<td>0.19</td>
<td>0.07</td>
<td>1.1</td>
<td>23</td>
<td>0.36</td>
<td>2.4</td>
</tr>
<tr>
<td>Fromage frais</td>
<td>100</td>
<td>Tr</td>
<td>0.05</td>
<td>0.02</td>
<td>0.04</td>
<td>0.4</td>
<td>0.13</td>
<td>0.1</td>
<td>1.4</td>
<td>15</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Gouda</td>
<td>245</td>
<td>145</td>
<td>0.24</td>
<td>0.53</td>
<td>0.03</td>
<td>0.3</td>
<td>0.05</td>
<td>0.08</td>
<td>1.7</td>
<td>43</td>
<td>0.32</td>
<td>1.4</td>
</tr>
<tr>
<td>Mozzarella</td>
<td>240</td>
<td>170</td>
<td>0.16</td>
<td>0.33</td>
<td>0.03</td>
<td>0.31</td>
<td>0.08</td>
<td>0.09</td>
<td>2.1</td>
<td>19</td>
<td>0.25</td>
<td>2.2</td>
</tr>
</tbody>
</table>
Cheese is an important dietary source of several minerals, in particular calcium, phosphorus and magnesium (Table 3). A 100-g serving of hard cheese provides approximately 800 mg calcium. However, acid-coagulated cheeses, e.g., Cottage, contain considerably less calcium than rennet-coagulated varieties (Renner, 1987). Bioavailability of the calcium from cheese is equivalent to that from milk. Reports show that 22.9, 26.7 and 25.4% of total calcium was absorbed from cream cheese, whole milk and yoghurt, respectively. While the aetiology of osteoporosis is very complex, it appears that adequate calcium intake during childhood and in the teenage years is important in assuring the development of high-peak bone mass. Maximizing bone mass early in life is considered to be a major preventative factor against the development of osteoporosis in later years. Cheese has a potential role in supplying extra, highly bioavailable, calcium.

Dairy products, including cheese, contribute little dietary iron (Table 3). Iron deficiency is commonly observed in both developing and developed countries. Hence, there has been interest in fortifying dairy products with iron to enhance their nutritional value. Cheddar and processed cheese have been successfully fortified with iron.

NaCl serves several important functions in natural and processed cheeses. A wide range of sodium levels are found in cheese due to different amounts of salt added during cheese making (Table 3). In general, the salt content of natural cheeses tends to be lower than that of many processed cheeses.

<p>| | | | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Parmesan</td>
<td>345</td>
<td>210</td>
<td>0.25</td>
<td>0.7</td>
<td>0.03</td>
<td>0.44</td>
<td>0.12</td>
<td>0.13</td>
<td>1.9</td>
<td>12</td>
<td>0.43</td>
<td>3.3</td>
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<tr>
<td>Processed cheese*</td>
<td>270</td>
<td>95</td>
<td>0.21</td>
<td>0.55</td>
<td>0.03</td>
<td>0.28</td>
<td>0.1</td>
<td>0.08</td>
<td>0.9</td>
<td>18</td>
<td>0.31</td>
<td>2.3</td>
<td></td>
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<tr>
<td>Ricotta</td>
<td>185</td>
<td>92</td>
<td>N</td>
<td>0.03</td>
<td>0.02</td>
<td>0.19</td>
<td>0.09</td>
<td>0.03</td>
<td>0.3</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Roquefort</td>
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<td>10</td>
<td>N</td>
<td>0.55</td>
<td>0.04</td>
<td>0.65</td>
<td>0.57</td>
<td>0.09</td>
<td>0.4</td>
<td>45</td>
<td>0.5</td>
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<tr>
<td>Stilton</td>
<td>355</td>
<td>185</td>
<td>0.27</td>
<td>0.61</td>
<td>0.03</td>
<td>0.43</td>
<td>0.49</td>
<td>0.16</td>
<td>1.0</td>
<td>77</td>
<td>0.71</td>
<td>3.6</td>
<td></td>
</tr>
</tbody>
</table>

N, the nutrient is present in significant quantities but there is not reliable information on the amount; Tr, trace

**Minerals**

Cheese is an important dietary source of several minerals, in particular calcium, phosphorus and magnesium (Table 3). A 100-g serving of hard cheese provides approximately 800 mg calcium. However, acid-coagulated cheeses, e.g., Cottage, contain considerably less calcium than rennet-coagulated varieties (Renner, 1987). Bioavailability of the calcium from cheese is equivalent to that from milk. Reports show that 22.9, 26.7 and 25.4% of total calcium was absorbed from cream cheese, whole milk and yoghurt, respectively. While the aetiology of osteoporosis is very complex, it appears that adequate calcium intake during childhood and in the teenage years is important in assuring the development of high-peak bone mass. Maximizing bone mass early in life is considered to be a major preventative factor against the development of osteoporosis in later years. Cheese has a potential role in supplying extra, highly bioavailable, calcium.

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NaCl serves several important functions in natural and processed cheeses. A wide range of sodium levels are found in cheese due to different amounts of salt added during cheese making (Table 3). In general, the salt content of natural cheeses tends to be lower than that of many processed cheeses.
Table 3 Mineral content of selected cheeses, mg/100 g (Holland et al., 1989)

<table>
<thead>
<tr>
<th>Cheese Type</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>P</th>
<th>Fe</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brie</td>
<td>700</td>
<td>100</td>
<td>540</td>
<td>27</td>
<td>390</td>
<td>0.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Camembert</td>
<td>650</td>
<td>100</td>
<td>350</td>
<td>21</td>
<td>31</td>
<td>0.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Cheddar (normal)</td>
<td>670</td>
<td>77</td>
<td>720</td>
<td>25</td>
<td>490</td>
<td>0.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Cheddar (reduced-fat)</td>
<td>670</td>
<td>110</td>
<td>840</td>
<td>39</td>
<td>620</td>
<td>0.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Cottage cheese</td>
<td>380</td>
<td>89</td>
<td>73</td>
<td>9</td>
<td>160</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Cream cheese</td>
<td>300</td>
<td>160</td>
<td>98</td>
<td>10</td>
<td>100</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Danish blue</td>
<td>1260</td>
<td>89</td>
<td>500</td>
<td>27</td>
<td>370</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>Edam</td>
<td>1020</td>
<td>97</td>
<td>770</td>
<td>39</td>
<td>530</td>
<td>0.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Emmental</td>
<td>450</td>
<td>89</td>
<td>970</td>
<td>35</td>
<td>590</td>
<td>0.3</td>
<td>4.4</td>
</tr>
<tr>
<td>Feta</td>
<td>1440</td>
<td>95</td>
<td>360</td>
<td>20</td>
<td>280</td>
<td>0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Fromage frais</td>
<td>31</td>
<td>110</td>
<td>89</td>
<td>8</td>
<td>110</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Gouda</td>
<td>910</td>
<td>91</td>
<td>740</td>
<td>38</td>
<td>490</td>
<td>0.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Mozzarella</td>
<td>610</td>
<td>75</td>
<td>590</td>
<td>27</td>
<td>420</td>
<td>0.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Parmesan</td>
<td>1090</td>
<td>110</td>
<td>1200</td>
<td>45</td>
<td>810</td>
<td>1.1</td>
<td>5.3</td>
</tr>
<tr>
<td>Processed cheese*</td>
<td>1320</td>
<td>130</td>
<td>600</td>
<td>22</td>
<td>800</td>
<td>0.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Ricotta</td>
<td>100</td>
<td>110</td>
<td>240</td>
<td>13</td>
<td>170</td>
<td>0.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Roquefort</td>
<td>1670</td>
<td>91</td>
<td>530</td>
<td>33</td>
<td>400</td>
<td>0.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Stilton</td>
<td>930</td>
<td>130</td>
<td>320</td>
<td>20</td>
<td>310</td>
<td>0.3</td>
<td>2.5</td>
</tr>
</tbody>
</table>

There is considerable evidence that high sodium intake contributes to hypertension in a susceptible minority (20%) of individuals who are genetically salt sensitive. Unfortunately, there is no simple diagnostic test to identify salt-sensitive individuals. Hence, dietary guidelines for the general public usually recommend that salt intake be restricted. However, it is important to note that even in countries with a high consumption, cheese contributes only about 5-8% of total sodium intake (Renner, 1987).

**Conclusion**

Cheese contains most of the nutrients of milk in concentrated and predigested form and also cheese as a highly versatile dairy ingredient that can be used directly in an array of culinary dishes, formulated food products, and readymade meals. In these applications, added cheese performs a number of functions; it contributes to structure, texture, flavor, mouth-feel, melt properties and nutrition.

**References**

TECHNOLOGICAL ADVANCES IN WHEY-BASED FERMENTED BEVERAGES

Sathish Kumar, M.H.

Introduction

Whey constitutes about 85–90% of the milk volume used for transformation into ripened cheese, and it retains about 55% of the milk nutrients. Preparation of one kg of paneer requires 5-6 liters of whole milk and about 4-5 liters of whey is produced as a by-product. Liquid whey is composed of lactose (5%), water (93%), proteins (0.85%), minerals (0.53%) and a minimum amount of fat (0.36%). Whey proteins have high biological value superior to other proteins such as those of egg, soy and caseins of milk (Smithers, 2008) mainly due to the high content of branched-chain essential amino acids (isoleucine, leucine and valine). These amino acids stimulate specific intracellular pathways associated with muscle protein synthesis (Katsanos et al., 2006) and may play a role in the hormonal response to feeding as stimulate insulin secretion (Calbet and MacLean, 2002). In addition to this, whey provides excellent growth medium for Lactic Acid Bacteria to ferment lactose in whey to form lactic acid and it is a good thirst quenching properties.

Whey proteins have increasingly made their way into human foods. For many years, whey was considered a by-product of cheese manufacture and was seen as an environmental pollutant. The main food usage was as animal feed, although dried whey was sometimes used as filler in human foods. From nutritional perspective whey proteins are important because they are highly digestible, provide all essential amino-acid, and are relatively cystine-rich. In fact, the major bovine whey proteins, β-lactoglobulin, α- lactalbumin, immunoglobulins, and bovine serum albumin contain two, four, four and 17 cystine per molecule, respectively. Cystine is the disulphide form of cysteine. It can be reduced to two moles of cysteine by cells for use in various processes including synthesis of the antioxidant glutathione.

Utilizing whey to make fermented whey beverages such as carbonated whey drinks, whey wine, beer like products and low alcoholic beverages appear to be most economical and viable process. Whey has been found to be a suitable growth medium for the proliferation of selected strains of lactic acid bacteria and yeast. In India there has been a tremendous increase in the production of cheese and coagulated milk products especially paneer and chhana resulting in a proportionate increase in whey.
Emerging health benefits of whey

Whey fractions are valuable for the food, dietary supplements, sports nutrition and nutraceutical industries. Commercially speaking, the most promising fractions are lactoferrin, lactoperoxidase, colostrum / immunoglobulin G (Ig G), α- lactalbumin, β-lactoglobulin and glycomacropeptides. Protein fractions such as lactoferrin, lactoperoxidase, α-lactalbumin, β-lactoglobulin, colostrum/Ig G, and glycomacropeptides are relatively new products on the market and are gaining in importance.

The global market for whey products is estimated to be worth around € 2.36 billion per year, of which the commodities market still represents the major part. Lactose, permeate, delactosed whey powder, sweet whey powder, acid whey powder, demineralised whey powder and whey protein concentrate account for around 85 per cent of this at € 2 billion.

Whey beverages

Various types of whey beverages have been developed by utilizing whey with or without fruit juice or milk. The Federal Republic of Germany in 1987 specified that whey drink must contain, in solid or liquid form, more than 51 per cent of whey constituents and may contain colouring, food stuffs with protein products and β-galactosidase

- Fruit flavoured whey beverages
- Enriched whey drinks
- Carbonated whey beverages
- Alcoholic whey beverages
- Whey milk beverages
- Fermented whey beverages
- Fermented whey milk beverages

Fermented whey beverages

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. Fermented milk products have been reported to have therapeutic properties like 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 absorbed in human digestive system.
In India, *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. Kumar *et al.* (1987) developed a *lassi*-type cultured beverage from cheese whey. Skim milk and cheese whey were heated and acidified with HCl (pH 4.5) to obtain the curd. The mixture was homogenized, pasteurized, cooled and inoculated with LF 40 culture. After 16 h incubation, sugar and synthetic pineapple flavour were added to obtain final *lassi*. *Lassi*-like beverage was developed using *paneer* whey and buffalo milk with pectin and CMC as stabilizers. After neutralization, *paneer* whey was mixed with standardized buffalo milk, followed by addition of stabilizer, heating, cooling, inoculation with mixed *dahi* culture (NCDC167) @ one percent and incubation at 30°C per 14 to 16 h. *Dahi* was then blended along with sugar syrup and flavour (Mittal, 2003). Kumar (2004) developed *lassi*-like beverage from rennet whey. Rennet whey and buffalo milk were admixed in definite proportion and this mixture was added with a stabilizer blend containing pectin, CMC and trisodium citrate followed by hydration, heating and cooling. Starter culture was added and the mixture was incubated for 12 h. The coagulum was blended after the addition of cooled sugar syrup and flavour. The *lassi*-like whey-based beverages developed as above 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 utilization of large quantities of whey. The product is also amenable to UHT-treatment and has a shelf life of over six months.

The process of Acidowhey making has been developed at NDRI, Karnal. In this process, whey collected from paneer, cheese, chhana or casein manufacturing is subjected to separation to remove fat and traces curd particles. Clarified whey is heat treated to 85-90°C for 10 minutes followed by cooling to 40°C. Then, inoculated with an active culture of *L. acidophilus* and incubated at 39±10°C and incubate for 20-22 hr. After incubation, fermented whey is clarified to remove precipitated cellular mass, whey protein and minerals formed during the process. Sugar is then added to the product @10-12% in the form of 50% sugar syrup which had been earlier pasteurized. At this stage desirable amount of flavor is added. The beverage is chilled to 4°C and packaged.

**Sensory qualities of fermented whey based beverages**

From the consumer’s point of view, fermented whey based beverages should be homogeneous in visual and in textural terms. Due to the low total solid content of liquid whey (approximately 6 %, by weight), the mouthfeel of whey-based beverages is poor and watery in comparison with fermented milk, thus requiring either the use of exopolysaccharide-producing starter cultures or the addition of hydrocolloids. Whey has also an unappealing taste, a relatively high lactose-glucose ratio and excessive acidity, especially if is acid whey. Therefore numerous
procedures have been developed for improving its characteristics aiming to enable its direct utilization in human nutrition. (Djurić et al., 2004)

**Functional fermented whey beverages**

Micaela et al. (2010) showed that WPC fermentation by rationally selected lactic acid bacteria might be used for developing functional beverages with improved characteristics such as reduced Beta Lactoglobulin (BLG) content and increased branched-chain essential amino acids. Fermentation of WPC by LAB could be an interesting alternative for the production of dairy functional foods with high nutritional content since whey proteins are known to have a high biological value. Macedo et al. (1999) prepared low cost, probiotic whey milk beverage using buffalo milk cheese whey, cowskin milk and soymilk.

Adrian Hernandez et al. (2007) made probiotic whey drink. *Lactobacillus reuteri* (2.8X10⁸ CFU.ml) and *Bifidobacterium bifidum* (4.7X10⁸ CFU/ml) was inoculated into reconstituted whey containing sucrose and pectin in order to prepare a fermented probiotic product. Inoculation levels were: 0.5, 1 or 2 % for *Lactobacillus reuteri* and 0.5 or 1 % for *Bifidobacterium bifidum*. The treatment with the highest bacterial counts and sensory scores was selected and stored at 4 °C for 30 days. The beverage fermented for approx. 11 h and prepared with 2 % *Lactobacillus reuteri* and 0.5 % *Bifidobacterium bifidum* met the probiotic criterion by maintaining both bacterial populations at counts greater than 10⁶ CFU/ml for the whole storage period. Titratable acidity and pH values as well as sensory properties did not change appreciably during the first 14 days of storage. At the end of the storage period (30 days), slight acidification was detected, although the beverage still retained an acceptable flavour.

**Conclusion**

There is an increasing trend in consumption of fermented whey drinks containing beneficial lactic acid bacteria and other cultures with probiotic properties in recent years. There has been recently a widespread increase in consumption of lactic beverages, such as drinking yoghurt, fermented milk products and milk-like drinks of which whey-based beverages constitute an emerging segment of non-conventional dairy products. Awareness on probiotics also driving the formulations using whey to couple nutritionally rich product with health enhancing attributes. Dairy industries in India, concentrating on utilization of whey in traditional dairy products like lassi and buttermilk to increase profitability and to provide good nutrition to the consumers.

**References**


Introduction

Food is consumed as a source of energy/nutrition for regular body metabolism. Developments in industrialization, urbanization and mechanization have resulted in dramatic change in dietary pattern and lifestyle of consumers. This in turn has increased occurrence of chronic non-communicable and communicable diseases such as obesity, diabetes mellitus, cardiovascular diseases, hypertension and stroke, cancer, and gastrointestinal infection. Incorporation of probiotics and prebiotics along with herbal components provides an opportunity to improve functionality of foods for their use for prevention or treatment. Fermented milk products have always been a choice of innovation to fulfil increased consumer preference for newness in functional dairy foods. Yoghurt, counterpart of Dahi, is extensively consumed by people of all age groups over the globe as part of their daily diet. However, dairy products do not always consist of probiotics, prebiotics and herbal components to harness their synergistic effect. Health conscious consumers also do not give much importance to foods with synthetic/chemical preservatives. Hence, incorporation of herbs or their active components such as essential oils (Eos) could be an effective strategy to improve functionality of milk and milk products w.r.t. health benefits, food safety and bio-preservation. This would also fulfil the needs of green consumerism. Therefore, it is highly desirable that probiotics, prebiotics and herbs used for the preparation of functional fermented dairy foods should be compatible to each other. These components should improve therapeutic quality without any adverse effect on sensory and rheological attributes. At present, there is hardly any proven synbiotic herbal fermented dairy food available in the market. Hence, it is imperative to explore enrichment of yoghurt with health promoting components (probiotic, prebiotic and essential oils) for its value addition w.r.t. therapeutic, food safety and shelf life enhancement.

India is known for its ancient civilization and renowned traditional medicinal knowledge “Ayurveda” unparalleled in the world for its minimum side effects as compared to allopathic drugs. As per World Health Organization estimates, 70% of the whole world and 85% population of developing countries depend on traditional knowledge for their healthcare needs. About 25 percent of modern medicines of 21st century have plant derivatives as their active ingredients or principles (WHO, 2002). Amongst, the herbal treasure of India Tulsi or Holy basil (Ocimum sanctum Linn.),
Mentha piperita, Cinnamon, Licorice etc., posses therapeutic and antimicrobial potentials against food spoilage and pathogen.

The global functional food market has emerged from a niche to a mainstream market category and continues to be a dynamic and growing segment of the food industry. World functional foods market has grown to US$167 billion in 2010 from US$ 7.63 billion in 2004 with composite annual growth of approximately 5% of total food expenditures in the developed world.

**Terminology related to health foods**

**Functional foods**

“That can beneficially affect one or more target functions in the body, beyond adequate nutritional effect, in a way relevant to an improved state of health and well being and/or reduction of risk of disease” (Stanton et al., 2005). Dairy products, particularly those containing probiotics, prebiotics i.e. synbiotics are most popular in this category of foods. A food is considered functional if it meets one of the following criteria:

- contains a food component (being nutrient or not) which affects one or a limited number of function(s) in the body in a targeted way so as to have positive effects,
- has physiological or psychological effect beyond the traditional nutritional effect.

**Probiosis**

“The positive effect of consumption of fermented dairy products with culture of lactic acid bacteria (LAB) on the equilibrium of intestinal microflora” (Tomasik and Tomasik, 2003). The benefits of consumption of these organisms comprise maintenance of gut health, increased bio-accessibility of lipids and proteins, reduced allerginicity of foods.

**Probiotics**

“Live microorganisms which, when consumed in adequate amounts confer health benefits on the host” (WHO, 2002). Lactobacillus and Bifidobacterium, the natural inhabitants of complex gastrointestinal tract micro-biota of human and warm blooded animals are extensively used as probiotics. These microorganisms play an important role in modifying the human health in addition to the regular nutrition, bio-preservation and safety of foods by inhibiting spoilage and pathogenic microorganisms.

**Prerequisite for probiotic cultures**

Probiotic cultures should be selected on the following criteria, irrespective of the intended host or site of application:

- Survival in the environmental conditions in the intended host.
• Proliferation and colonization under the host environmental condition.
• Compatible with the host immune system, and non-inflammatory.
• Immuno-stimulatory for the mucosal immune system.
• Production of antimicrobial metabolites and inhibitory against food spoilage as well as pathogenic micro-organisms.
• Non-pathogenic, non-toxic, non-allergic, non-mutagenic or anti-carcinogenic, even in immune-compromised hosts.
• Genetically stable, non-plasmid transfer.
• Technologically suitable for process applications.
• Potential for delivery of recombinant proteins and peptides.
• Desirable metabolic activity and antibiotic resistance / sensitivity.

Prebiotics

"Non-digestible substances that exert some biological effect on humans by selective stimulation of growth or bioactivity of beneficial microorganisms either present or therapeutically introduced into the intestine" (Roberfroid, 1998). Gibson (2000), suggested that fructo-oligosaccharide viz., inulin, galacto-oligosaccharide, lactulose, lacto-sucrose, isomaltol-oligosaccharides, gluco-oligosaccharides, xylo-oligosaccharides, soya-bean oligo-saccharides have prebiotic potential. Although, there is no daily-recommended dose of prebiotics, however, 4-20 g/d is required to show health benefits (Tuohy et al., 2003).

Prebiotic criteria: A food ingredient is classified as a prebiotic if it fulfils the following criteria:
• Neither be hydrolyzed, nor be absorbed in the upper part of the gastrointestinal tract.
• Selectively fermented by one or a limited number of potentially beneficial bacteria commensal to the colon, e.g. bifidobacteria and lactobacilli,
• Must be able to alter the colonic microflora towards a healthier composition, e.g. increase in saccharolytic and reduction of putrefactive microorganisms

Synbiotics

"Mixture of probiotics and prebiotics that beneficially affects the host by improving survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria, and thus improving host welfare" (Tuohy et al., 2003). Experiments have provided evidence that synbiotics perform better than either probiotics or prebiotics alone.

Developments & technological challenges in synbiotic herbal yoghurt preparation

According to FAO/WHO standards, yogurt is "the coagulated milk product obtained by lactic acid fermentation through the action of Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus" (Krasaeuko et al., 2005). The variety of functional foods that can be developed is driven by the imagination of scientists, the
perceived benefits, and the willingness of consumers to pay for those benefits. Functional food poses few challenges; since most of the probiotics are sensitive to food environment (acidity & dissolved oxygen), survival during passage through harsh gastrointestinal environment (low pH & bile salt conc.) and storage as well as short shelf-life. Addition of prebiotics and herbal products to fermented dairy foods also affect their sensory and rheological quality. Health conscious consumer demands natural safe foods with improvised health benefits. This provides new opportunity for food scientists to address these challenges. The most important textural characteristics of yoghurt, firmness and ability to retain water, which results in a smooth viscous gel, are a major concern to manufacturers of low-fat yogurt. These characteristics, related to the gel structure, can be improved by manipulation of dairy starter and fat replacer especially low fat yoghurt the later can be a prebiotic to obtain smooth mouthfeel of the product. These challenges can be meted out with the following manipulations:

Table 1: Health benefits associated with synbiotic foods.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Health benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alleviation of lactose intolerance</td>
</tr>
<tr>
<td>2</td>
<td>Improvement in Ca, Fe and Mg absorption</td>
</tr>
<tr>
<td>3</td>
<td>Anticancer effect</td>
</tr>
<tr>
<td>4</td>
<td>Cardiovascular health</td>
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<tr>
<td>5</td>
<td>Cholesterol assimilation</td>
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<tr>
<td>6</td>
<td>Modulation of immune function</td>
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<tr>
<td>7</td>
<td>Constipation alleviation</td>
</tr>
<tr>
<td>8</td>
<td>Treatment of diarrhoea</td>
</tr>
<tr>
<td>9</td>
<td>Prevention / treatments of infections</td>
</tr>
<tr>
<td>10</td>
<td>Increase in nutrient bioavailability</td>
</tr>
<tr>
<td>11</td>
<td>Regularisation of intestinal flow</td>
</tr>
<tr>
<td>12</td>
<td>Production of vitamins</td>
</tr>
</tbody>
</table>

- **Acid sensitivity** is principal factors for poor viability of probiotic cultures particularly bifidobacteria in fermented dairy foods. However, microencapsulation technique can be used to improve viability of acid sensitive cultures in food systems.

- **Oxygen sensitivity** is of particular relevance to bifidobacteria as they are strict anaerobes. Toxic effects of oxygen can be overcome; milk may be deaerated prior to fermentation. Alternatively, use of impermeable packaging may eliminate the toxic effects of oxygen during product storage. Addition of reducing agents such as cysteine or oxygen scavengers such as ascorbic acid and selection of oxygen tolerating strains may also improve the tolerance of probiotic cultures to oxygen sensitivity.

- **Processing parameters** such as thermo tolerance is an important parameter when considering microbial survival in food processes such as spray-drying. Within the genera most often employed as probiotics, certain strains and
species are more heat tolerant than others e.g. “thermophilic” lactobacilli (Kandler and Weiss, 1989).

- **Exo-polysaccharides producing probiotic lactic starters** could be used to improve rheological attributes of yoghurt as this has better water binding capacity, which decreases the product's susceptibility to syneresis (Amatayakul et al., 2006). There is no direct correlation between viscosity and quantity of EPS produced however, it is opined that combination of ropy and non-ropy starter cultures give better textural property. Amount and type of EPS production in milk is strain/species dependent and is influenced by cultural growth conditions. Thus, the functionality of EPS starters depends on the nature of polysacc-haride, its composition, structure, type of linkage, branching and side groups as well as its interaction with other constituents of milk (Lin and Chang Chien, 2007). However, the interaction of EPS with other milk components has not been studied as yet to any great detail nor is the influence of EPS on the rheological and therapeutic properties of yogurts containing probiotics and fat replacers investigated.

- **Fat replacer** is an ingredient that can be used to provide some or all of the functions of fat, yielding fewer calories than fat. These are also used to overcome textural defects in low-fat/no-fat yoghurt. The major categories of fat replacers include carbohydrate based - (Avicel, Opta, Raftline), protein based - (Simplessse, Dairy-Lo, Versagel) and fat based - (SALATRIM, OLESTRA) replacers. Among these, inulin has gained popularity as it provides physiological benefits and lower calorific value (Roberfroid, 1999) and thus can be used in foods designed for weight management.

- **Secondary metabolites** of lactic acid fermentation such as B vitamins and bioactive peptides (proteins with biological functions or physiological effects) have health promoting properties. Physiologically active peptides are also produced from several food proteins during gastro-intestinal digestion and fermentation of food by LAB, their oral administration affect major body systems i.e. cardiovascular, nervous, gastrointestinal and immune systems depending upon inherent amino acid composition and sequence. Among these, peptides with blood pressure lowering effects have received special attention and considerable significance is being attached to the role of diet in the prevention and treatment of the disease (López-Fandiño et al., 2006).

**Health benefits of Tulsi (Ocimum sanctum)**

This is most important medicinal herb ever known for its miraculous and medicinal values. The health benefits include skin care, dental care, relief from respiratory disorders, asthma, fever, lung disorders, heart diseases, stress, reduce labor pain, destroy rabies germs, treat gastroenteritis, mumps, cholera, whooping cough, measles, rheumatism, nausea, septic, urinary, genital infections, etc. Recent, reports also indicate its inhibitory activity against growth of HIV and carcinogenic cells. It protects from radiation poisoning and also heal up damages from it. It acts as a vaccine against pox if consumed regularly. It is anti-carcinogenic and quite effective
in healing, nearly all types of cancer and tumors. Dried leaves are used as insect-repellant by mixing with food grains.

**Essential oils**

Essential oils are aromatic liquids obtained by extraction or steam distillation (commercial production) from plant parts (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). The term ‘essential oil’ was coined in 16th century by the Swiss reformer of medicine, Paracelsus von Hohenheim; who named the effective component of a drug *Quinta essentia*. It is well established that some essential oils possess antimicrobial properties against food pathogens and spoilage organisms.

**Chemical composition:** Essential oils are a group of terpenoids, sesquiterpenes and possibly diterpenes with different groups of aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones. Plant extracts principally constitute essential oils (85%) which exhibit antimicrobial activity and can be characterized by gas chromatography or mass spectrometry. The phenolic components of essential oil are mainly responsible for their antimicrobial activity. The composition is affected by harvesting season and geographical sources of herbs.

**Antimicrobial activity:** *Padmottara Purana* (Indian mythological book) asserts that a house where garden of *Tulsi* exists, is itself a centre of pilgrimage; neither servants of *Yama* (the lord of death) nor disease can enter there and wherever fragrance of *Tulsi* goes, the air gets purified (Ghosh, 1995). This statement seems to have some relevance because essential oil forms specific fragrance, volatile in nature and capable of killing of variety of microbes. Aqueous and acetone extracts of *Ocimum sanctum* are effective against fungi, Gram +ve and Gram -ve microorganisms including multi-drug resistant *Neisseria gonorrhoeae*.

**Antimicrobial activity mechanism:** Essential oils damage bacterial cell due to disruption of cytoplasmic membrane, proton motive force (PMF), electron flow, active transport and coagulation of cell contents. They affect microbial cells by multi target mechanism due to presence of large number of different groups of chemical compounds (Fig 1). Antimicrobial mechanism of oil depends upon various factors like method of extraction, intrinsic and extrinsic properties of food such as pH, fat, protein content, antioxidants, preservatives, incubation time and temperature and packaging procedure.
Efficacy of essential oils: This depends on number of factors such as method of extraction, volume of inoculum, stage of growth phase, culture medium used, and intrinsic or extrinsic properties of the food. Generally higher concentrations are required to achieve the same effect in foods as compared to laboratory media because food stuff provides microbial resistance due to greater availability of nutrients in foods.

Techniques for antimicrobial activity determination: Usually antibacterial activity of essential oils is quantified on the basis of MIC (minimal inhibitory concentration) and MBC (minimum bactericidal concentration). MIC is the “lowest concentration that inhibits visible growth of test organism”. MBC is “concentration where 99.9% or more of the initial inoculum is killed” (NCCLS, 2000). Other methods such as disc-agar-diffusion method, drop-agar diffusion method and direct-contact technique in agar are the usual techniques in the screening approach.

Need for herbs in dairy products

The growing worldwide data indicates a strong consumer demand for safe and high quality foods. As a consequence, natural antimicrobial substances are receiving a good deal of attention for their usage for controlling undesirable microbes as alternative to chemical preservatives. Herbs provide natural antioxidants; improve sensory attributes and also acts as natural bio-preservatives. By incorporation of herb into dairy foods would result in value added product diversification in dairy
industry. Fortification of dairy products with herbs would provides improved functional properties w.r.t. food safety and therapeutic over conventional nutrition.

**Safety aspects of essential oils**

Applications of traditional and natural antimicrobials have been approved by regulatory agencies in the U.S, recently and given the GRAS status (Tajkarimi, 2010). However, no such regulations have been approved in India till date. Essential oils can widely be applied as antimicrobials in foods if it doesn’t alter its taste and non-genotoxic. Flavouring substances mainly carvacrol, carvone, cinnamaldehyde, citral, p-cymene, eugenol, limonene, menthol and thymol are considered as safe. As they do not present any risk to the health of consumer. Some oils used in the fields of medicine, paramedicine and aromatherapy have been shown to exhibit spasmyltic or spasmogenic properties, although these are difficult to associate with a particular component. In spite of the fact that a considerable number of EO components are of GRAS and/or approved food flavourings, still some research data indicate their irritation and toxicity.

**Food safety and shelf-life enhancement by essential oil**

Perishable food products require protection from spoilage during preparation, storage and distribution to give them desired shelf life and food safety. Addition of essential oils in food products improve taste and food safety and enhance shelf-life because of their antimicrobial nature. Mint oil at 5–20 µl/g is effective against *S. enteritidis* in low fat yoghurt. Essential oils of clove, cinnamon, bay and thyme were tested against *L. monocytogenes* and *Salmonella enteritidis* in soft cheese; clove oil was found more effective against *Salmonella enteritidis* in full-fat cheese than in cheese slurry. Extract of mango seed kernel could reduce total bacterial count, inhibit coliform growth, exert remarkable antimicrobial activity against *Escherichia coli* strain and extended shelf life of pasteurized cow milk (Abdalla *et al.*, 2007). Application of nisin with carvacrol or thymol has been positively effective against *Bacillus cereus* with temperatures increasing from 8 to 30°C (Burt, 2004). Application of nisin with rosemary extract enhanced the bacteriostatic and bactericidal activity of the nisin.

**References**

Abstract: The function of writing is to communicate, and any writing that makes for clear and accurate communication is a good writing. Technical Writing is the art of recording information on specialized/technical fields accurately and effectively. Technical Writing is objective in content and systematic in form. The nature of the subject, the purpose of the report and the reader for whom the report is written determine the form and structure of the report. Good Technical Writing depends upon the correct use of language and a good style of writing. Technical writing uses structure, rather than the physical presence of the writer, to achieve clarity. Technical Writing communicates through the precision of its diction, the orderliness of its sentence and paragraph structure, and the relative fullness of its detail. This could be possible only through simple, direct and plain style using simple language. Every written communication should be carefully planned and constructed keeping the targeted reader in mind.

Communication is a process involving transferring of information and sharing of ideas from one person to the other. Besides the core competence and knowledge in one’s specialized field, communication skills contribute a lot to the success of an individual in any organization. These skills form an integral part of leadership and managerial skills, one of the essential elements required for developing competence needed for career success in the 21st Century. This is the only completely portable skill, used in every relationship and required regardless of any career path. Language and written documents facilitate the transfer of information and knowledge through time and space.

Technical writing/reporting

Technical Writing/Reporting is a specialized branch of the field of communication. Technical Writing is used in all fields of science, technology, agriculture, engineering and social sciences. Any branch of knowledge requiring a systematic study involves the use of scientific and technical writing for the purpose of recording and reporting information. This is the art of recording information on specialized fields accurately and effectively and passing it on to those who have to use and process it. Technical writing uses structure, rather than the physical presence of the writer, to achieve clarity. It has to be clear, simple and well ordered communication to transmit the facts and findings.
Importance of technical reporting

Students: The typical undergraduate student regards the writing of reports as a dull and superfluous chore. Consequently, he has little desire for instruction in technical writing. One of the main reasons for this state of affairs is that the undergraduate—particularly in his/her earlier years—seems to have very little to say. As he programs through college and on into graduate school or industry, he develops a body of knowledge. At some time in his career, he acquires some information or some idea that he wants to pass on to others. This is when he needs to acquire skills in technical reporting.

Big organizations: The complexity of an organization increases exponentially with its size. And as the complexity goes up, soon too does the need for written records and communications. Only through a full exchange of information can the various divisions of large organization co-ordinate their efforts effectively.

Small organizations: But even a small organization has a vital need for accurate technical reporting. How was a special part fabricated last year? How was a test performed? What are the precautions to be observed with seldom used instrument? Written records furnish authoritative answers to many questions as these, and increase the efficiency of organization that maintains vigorous reporting procedure.

Scientific organizations: In many of the scientific organizations, particularly those doing experimental work or research, the young employee’s chief communication with his superiors is through his written (or oral) reports. Often the superior has no other criterion by which to judge an employee’s work. Moreover, these scientific organizations do nothing but investigation, testing, experimentation, or research. Their only tangible product is the report. If they are to have anything to show for their efforts, they must do thorough job of reporting.

Many industrial and research organizations nowadays place so much value on high quality reports that they maintain separate editorial departments to write technical report or to edit and polish them. Reports have achieved a recognized position of importance in our technological world.

Functions of technical writing

Technical Reporting is different from creative writing as it deals with scientific facts and does not present an imaginary view of reality. Scientific and Technical 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.

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-
The contents: The subject of the report primarily determines the nature of the contents. Report writing is meaningless when the writer is not clear about the subject of his report. However, the detailed aspects of the contents are determined by the purpose for which the report is written. Basic questions (5 Ws i.e. What, Why, Who, Where, When, and How) need to be answered satisfactorily. The answers depend on the usefulness of the information to the reader and his interest in the subject, the details of the work carried out, and the recommendations and suggestions you intend making and their implications.
There is no neat formula for the organization of technical reports. Each report must be organized to fit its own subject, its own purpose, its own audience. But a few general principles apply to most technical communications.

**Logical progression toward conclusions:** The material in any report should be presented in an order that leads logically towards a conclusion or conclusions. This doesn’t mean, of course, that everything in a lengthy report will aim at one final climax; the various sections of the report are organized so that each of them has its logical conclusions.

**The three parts:** Almost every technical communication should have three functional elements. This does not mean that it should be divided by boundaries into three distinct parts. But functionally it should have a beginning, middle and an end.

The beginning orients the reader and supplies him with background material, so that he will see how the subject of the paper fits into the general scheme of things. It prepares the reader for the main presentation of information—the middle. The beginning is often called **Introduction**, which states the purpose of the investigation and describes the basic scheme of the procedure or methods used. It orients the reader by supplying as much historical background as necessary and then describing the present problem. It may define the scope of the study, discussing limitations or qualifications.

**The middle** is usually the longest part of the report. It can be organized in many different ways:

- It tells what you did. (Description)
- It tells what you found out. (Results)
- It analyzes, interprets and discusses these results. (Discussion)

**The end** is sometimes labeled conclusions. It brings together the various subjects that have been discussed and shows their relationships with each other and with broader fields. This end section makes the exposition come to a logical and an obvious termination, rather than simply stop a note of detail. It ties a string around the bundle.

**Style of technical writing**

**Good technical writing** 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, capitalizations 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.
Choice of words

The primary objective of Technical Writing is to transmit information briefly, clearly and efficiently. This can be achieved only through simple, direct and unadorned 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. Abstract words tend to be general and vague. As a result, expressions that contain abstract nouns are less forceful, less direct, and less exact than their concrete counterparts.

Verbosity (Wordiness)

For simple, clear style, eliminate from the writing every word that does not contribute to the meaning or clarity of your message. Long-winded phrases and superfluous words should be avoided. For example, the phrase “because of the fact that” can be substituted with the word “because”. On the other hand, so many words should not be eliminated that the writing reads like a telegram. If a word adds anything worthwhile to the meaning, grace, rhythm or emphasis in the sentence, it should not be omitted.

Jargon

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.

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.

The passive voice

In the passive voice, the subject is the receiver of an action rather than the doer of it. Passive voice is employed by writers when they want to evade or conceal the responsibility for someone’s behaviour. As the passive voice is sometimes vague and less economical than the active voice, good writers tend to avoid it except when it is genuinely useful. The passive voice may be preferable, for example, when the real
doer of an action is either unknown or, in the context of a discussion, relatively important.

**Subordination**

A common failing of technical writers is the expression of ideas of unequal importance in constructions that seem to give equal weight. 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.

**Conclusions**

Scientific and Technical Writing is objective in content and systematic in form. The primary objective of Technical Writing is to transmit information briefly, clearly and efficiently. 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. This could only be achieved through simple, direct and plain style using simple language. Every written communication has a specific purpose and a specific audience. It should be carefully planned and constructed keeping the reader in mind.
Process cheeses are characterized essentially by composition, water content and consistency; according to these criteria, three main groups may be distinguished: processed cheese blocks, processed cheese foods, and processed cheese spreads (Table 1). Other established sub-types of processed cheeses are processed cheese slices and smoked processed cheese. The first sub-type belongs to the category of processed cheese blocks while the second could be either block or spread. Processed cheese spread is spreadable at 21°C. The basic aim of processing is to blend natural cheese, water emulsifying salts and pasteurize the mixture to obtain an end product which has sufficient fluidity for convenient packaging and which possesses a longer shelf life. A comprehensive review of processed cheese products (Caric and Kalab, 1993) details the principles and techniques of cheese processing, the types and functions of emulsifying agents, microstructure of processed cheese, and the quality defects of microbial and physicochemical origin.

Pasteurized processed cheese products are produced by comminuting, melting and emulsifying into a smooth homogenous molten blend one or more natural cheeses and optional ingredients using heat, mechanical shear, and (usually) emulsifying salts. Optional ingredients permitted are determined by the product type (processed cheese, processed cheese food and processed cheese spread) and include dairy ingredients, vegetables, meats, stabilizers, emulsifying salts, flavours, colours, preservatives and water.

Many developments have taken place in the area of processed cheese all over the world. The changes have come into being in the selection of raw material, various emulsifying agents and their combinations, method of processing and equipments used and the enrichment of processed cheeses. The fact that there is a growing volume of literature on all aspects of processed cheese processing and quality from all parts of the world reflects, without doubt, an increasing interest in this versatile dairy product. Factors contributing to the continued growth and success of these products are as follows:

- They offer almost unlimited variety in flavour consistency, functionality (e.g., sliceability, meltability, flowability) and consumer appeal as a result of differences in formulation, processing conditions and packaging into various shapes and sizes.
• They cost less than natural cheese because they incorporate low-grade natural cheese and cheaper non-cheese dairy ingredients.
• They are adaptable to the fast-food trade.
• They have a relatively long shelf-life, and waste is minimal.

Manufacturing protocol for processed cheese products

Manufacture of processed cheese products involves the following steps:

Selection of natural cheese

Proper selection of natural cheese is of utmost importance for the successful production of processed cheese. In some countries, processed cheese manufactured from only one variety of cheese of different degrees of maturity are very popular, e.g., processed Cheddar cheese in the UK and Australia; Cheddar, Gruyere and Mozzarella in the USA and Canada; Emmental in Western Europe. More frequently, processed cheeses are produced from a mix of various natural cheese types. This result in easier processing and a better flavour balance. The most important criteria for cheese selection are: type, flavour, maturity, consistency, texture and acidity (pH). However, proper selection of good-quality natural cheese is not, by itself, a guarantee that the processed cheese will be of the high quality desired.

Table 1: Some characteristics of processed cheese types

<table>
<thead>
<tr>
<th>Type of product</th>
<th>Ingredients</th>
<th>Cooking temperature (°C)</th>
<th>Composition</th>
<th>pH</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed cheese block</td>
<td>Natural cheese, emulsifiers, NaCl, colouring</td>
<td>71-80</td>
<td>Moisture and fat contents corresponding to the legal limit of natural cheese</td>
<td>5.6-5.8</td>
<td>Kosikowski (1982)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80-85</td>
<td></td>
<td>5.4-5.6</td>
<td>Meyer (1973)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>74-85</td>
<td></td>
<td>5.4-5.7</td>
<td>Thomas (1977)</td>
</tr>
<tr>
<td>Processed cheese food</td>
<td>Same as above plus optional ingredients such as milk, skim milk, whey products, cream, albumin, skim milk cheese; organic acids</td>
<td>79-85</td>
<td>&lt;44% moisture, &gt;23% fat</td>
<td>5.2-5.6</td>
<td>Kosikowski (1982)</td>
</tr>
<tr>
<td>Processed cheese spread</td>
<td>Same as processed cheese food plus gums for water retention</td>
<td>88-91</td>
<td>40-60% Moisture &gt;20% Fat</td>
<td>5.2</td>
<td>Kosikowski (1982)</td>
</tr>
</tbody>
</table>

Blending

This operation is strongly influenced by the desired characteristics of the final products. According to Thomas (1977), general formulation of processed cheese (block-type) constitutes 70-75% of the mild cheese and 25-30% of semi-mature or mature cheese. For the production of processed cheese in slices, where a high content of elastic, intact (unhydrolysed) protein is necessary, this ratio is changed to: 30-40% young cheese, 50-60% mild cheese and only 10% mature cheese. Kosikowski (1982) suggests a similar blend composition: 55% young cheese, 35% medium aged and 10% aged cheese, in order to obtain optimum firmness and slicing qualities. However, if a processed cheese spread is to be produced, the principal raw material is semi-mature cheese of shorter structure, i.e. with partially
hydrolysed protein, e.g. 30% young cheese, 50% semi-mature cheese and 20% mature cheese. In addition to natural cheeses, various other dairy and non-dairy ingredients are used in the production of processed cheese spreads and processed cheese foods. Since, the quality of the final product is influenced considerably by all the components present in the blend, the non-cheese components must also fulfill certain qualitative and quantitative requirements. The most frequently used non-cheese ingredients are skim milk powder, casein, whey protein, coprecipitates, various whey products and milk fat products.

Although, ordinary whey powder is the most common whey product used in processed cheese, whey protein products with lower mineral and lactose contents are preferable, because they yield processed cheese with better flavours. Whey protein concentrates (WPC) of various composition, obtained by ultrafiltration (UF) are now available that are increasingly being used as ingredients in processed cheese. The advantages of whey protein addition to the blend have been described (Caric, 1991). German workers have reported (Gupta and Reuter, 1992) that WPC could be used to replace 20% of cheese solids in processed cheese food formulations. According to the process, a mixed blend of ground Cheddar cheese with 20% WPC, salt and water was heated by indirect steam to 45°C, sprinkled with 2.5% dry trisodium citrate and heated at 82°C for 3-4 min. The firmness of the processed cheese products decreased in a significant manner (P<0.01) and melting quality increased in a significant manner (P<0.01) with the increase in moisture over a wide range. Increasing the amount of WPC and trisodium citrate significantly improved firmness (P<0.01), but had a significant deleterious effect (P<0.01) on the melting quality of the products (Gupta and Reuter, 1993).

Numerous investigations have been carried out in order to develop new processed cheese blends with improved characteristics and/or which can be produced at a low cost. The manufacture of a high moisture, low fat processed cheese is covered by a US patent (Batz et al., 1993). The process involves heating a mixture of particulate skim milk curd, salt and an emulsifying salt to temperature above 160°C under agitation. Following the optional addition of an acid to the cheese blend, an aqueous mix consisting of milk protein, a texture modifying agent and water is added to achieve the high moisture low fat cheese blend. The end product has a moisture content of at least 50%. The manufacture of a virtually fat free processed cheese is described in another US patent (Davidson, 1993). It is based on a skim milk cheese also containing dried milk, dried whey and dried buttermilk, the fat content of the finished cheese being approximately 1.67%.

Acceleration of the ripening process in the production of 'natural' cheese destined for use in manufacture of processed cheese is particularly interesting, both technologically and economically. The successful production of a cheese base from reconstituted dried skim milk, which had been ultrafiltered/diafiltered to reduce the lactose content, is reported (Tamime et al., 1991). The cheese base contained 45.6%
TS, 31.4% protein, 4.7% minerals and 3.5% lactose with a pH of 5.3. Excellent microbiological quality was obtained and cheese ripening could be accelerated through the use of Savorase-A. The experimental processed cheese (Cheddar), containing no more than 40% cheese base, was of good quality.

Another method for obtaining the desired ripened cheese flavour in processed cheese is the incorporation of the newly developed EMC (enzymatically modified cheese) in the blend. Flavour intensity of EMC is increased about 10 to 30 times compared to the corresponding traditional cheese. Commercial preparations of Cheddar, Emmental, Parmesan and other modified cheese flavours are available, which give excellent results in processed cheese products.

**Processing**

Processing means heat treatment of the blend, by direct or indirect steam, under a partial vacuum and with constant agitation. There are two basic types of cooking device: (i) round double-jacketed kettle, up to 200 l, and (ii) tube-shape (about 4 m long, fitted with one or two mixings worms). Due to their greater versatility in production, kettles are most common in Europe, while in North America, where large-scale production of processed cheese slices is dominant, horizontal devices are preferred. If processing is performed batchwise, i.e. in a kettle, the temperature reached is 71-95°C for a period of 4 to 15 min (Table 1), depending on various parameters; this heating also provides a pasteurization effect. A newly programmed jacketed processor has been developed, which is used to grind, mix and process natural cheeses with other blend components, water and emulsifiers using steam injection and a vacuum, at 75°C for 5 min. The processor is programmed via a punch-card for blend formulation and cleaning-in-place. After cooling, the blend is discharged either by tilting the processor or by aseptic pumping to a packaging machine. When continuously processed, the blend is sterilized at 130-145°C for 2-3 s in a battery of stainless steel tubes (Kosikowski, 1982). Zimmermann (1982) patented a continuous process for simultaneous melting, homogenization and sterilization in processed cheese production without the application of pressure. A Japanese patent describes a new method for the post-processing heat treatment (to 100°C) of packed processed cheese, produced in the usual way. Chemical, mechanical and thermal parameters used in cheese processing are listed in Table 2.
Table 2: Chemical, mechanical and thermal parameters as regulating factors in the cheese processing procedures

<table>
<thead>
<tr>
<th>Process conditions</th>
<th>Processed cheese blocks</th>
<th>Processed cheese slice</th>
<th>Processed cheese spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw material</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average of cheese</td>
<td>Young to medium ripe, predominantly young</td>
<td>Predominantly young</td>
<td>Combination of young, medium ripe, overripe</td>
</tr>
<tr>
<td>Water insoluble N as a % of total N</td>
<td>75-90%</td>
<td>80-90%</td>
<td>60-75%</td>
</tr>
<tr>
<td>Structure Emulsifying salt</td>
<td>Predominately long, structure-building, not creaming, e.g. high molecular weight polyphosphate, citrate mixtures</td>
<td>Long, structure-building, not creaming, e.g. phosphate/citrate mixtures</td>
<td>Short to long, creaming, e.g. low and medium molecular weight polyphosphate (in portions)</td>
</tr>
<tr>
<td>Water addition</td>
<td>10-25% (All at once)</td>
<td>5-15% (all at once)</td>
<td>20-45% (in portions)</td>
</tr>
<tr>
<td>Temperature</td>
<td>80-85°C</td>
<td>78-85°C</td>
<td>85-98°C (150°C)</td>
</tr>
<tr>
<td>Duration of processing, min.</td>
<td>4-8</td>
<td>4-6</td>
<td>8-15</td>
</tr>
<tr>
<td>pH</td>
<td>5.4-5.7</td>
<td>5.6-5.9</td>
<td>5.6-6.0</td>
</tr>
<tr>
<td>Agitation</td>
<td>Slow</td>
<td>Slow</td>
<td>Rapid</td>
</tr>
<tr>
<td>Reworked cheese</td>
<td>0-2.0%</td>
<td>0</td>
<td>5-20%</td>
</tr>
<tr>
<td>Milk powder or whey powder, 5-12%</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Homogenization</td>
<td>None</td>
<td>None</td>
<td>Advantageous</td>
</tr>
<tr>
<td>Filling, min.</td>
<td>5-15</td>
<td>The quickest possible</td>
<td>10-30</td>
</tr>
<tr>
<td>Cooling</td>
<td>Slowly (10-12h) at room temperature</td>
<td>Very rapid</td>
<td>Rapidly (15-30 min) in cool air</td>
</tr>
</tbody>
</table>

References


Introduction

Imitation cheeses or cheese substitutes may generally be defined as products that are intended to partly or wholly substitute or imitate cheese, in which milk fat, milk protein, or both are partially or wholly replaced by non-milk-based alternatives, principally of vegetable origin. In the United States, an imitation cheese is defined as a product, which is a substitute for and resembles another cheese, but is nutritionally inferior. Imitation or substitute cheese products are made to resemble standardized or non-standardized cheese varieties, processed cheese, cheese foods and cheese spreads. The production of these cheese substitutes has received considerable attention, not only from an economical point of view, but also because the use of various types of high protein products like rennet or acid casein, caseinates or even soy protein isolates allows the production of products with a wide range of physicochemical and organoleptic properties. Hitherto developments in imitation cheese have been confined almost exclusively to the United States. Outside the United States, there is little specific legislation covering imitation cheeses. In imitation cheeses, the main emphasis at present is on the replacement of milk fat by vegetable fat. However, replacement of milk protein by vegetable protein is a likely future objective, depending on the technology (McCarthy, 1990). Cheese imitations are almost thirty per cent cheaper to manufacture than the real cheese. They seem to have a longer life and this makes them particularly suitable for certain uses like in the preparation of Pizzas and the manufacture of prepared dishes. Promoters of imitation cheese claim nutritional advantages compared with genuine cheese, i.e., unsaturated fatty acids, much lower or no cholesterol and lesser calories.

Imitation cheese types

There are few, if any, standards relating to permitted ingredients or manufacturing procedures for imitation cheese products. The products may be arbitrarily classified into three categories: analogue cheeses, filled cheeses and tofu, based on the ingredients used and the manufacturing procedure.

Cheese analogues

Analogue cheeses, which were introduced in the United States in the early 1970s, constitute by far the largest group of imitation or substitute cheese products. The manufacture of analogues of a wide variety of natural cheeses (e.g., Cheddar, Monterey Jack, Mozzarella Parmesan, Romano, Blue cheese and Cream cheese) and
pasteurized processed cheese products has been reported in the trade literature. The major products in USA are substitutes for or imitations of low-moisture Mozzarella, Cheddar and pasteurized processed cheddar. These products find application mainly as cheese topping for frozen pizza pie and as slices in beef burgers. Other applications include use in salads, sandwiches, spaghetti sprinkling, cheese sauces, cheese dips, and ready-made meals.

These products are made from vegetable fat, milk protein and various additives and flavouring components using the processed cheese technology. They have good melting properties, stretch and flow and are accordingly used in Pizza manufacture and cooking applications replacing the genuine Mozzarella. The trend is towards increasing utilization of rennet rather than acid casein. There have been developments in the use of soya and other vegetable proteins in the production of cheese analogues. Casein extended by the use of vegetable protein is also available, but reports indicate that, so far, it has not been feasible to include more than 5% vegetable protein as extender. Cheese analogues are also available as consumer processed cheese, processed cheese food and processed cheese spreads.

**Filled cheeses**

In this case, skim milk and vegetable fat are processed by methods that are essentially identical to those for regular cheese varieties. Reports suggest that these have not been as successful as cheese analogues. Products are claimed to have 30 to 90% less cholesterol and up to 50% fewer calories.

**Substitute cheese products based on tofu**

Tofu is a rubbery curd like material made from soya protein. It is derived from soya milk by a salting-out effect under specific conditions of pH. Soya milk is coagulable in the pH range of 5.0 to 4.5 and the so called silken tofu can be produced using Glucono-δ-lactone. A number of soya-based substitute cheese products have been introduced recently. The main ingredients in these products are tofu, casein and soya oil. It is claimed that the products contain no cholesterol, lactose, butter fat or artificial ingredients and have a shelf life of up to 100 days.

**Raw materials**

**Caseins and Caseinates**

Acid casein, sodium caseinate, calcium caseinate and rennet casein can be used as additives for processed cheese preparations and as raw material for products similar to processed cheese. Of these, calcium caseinate is mainly used in the manufacture of imitation cheeses according to recipes specially devised for this raw material. Casein derivatives have desired functional properties e.g. binding of fat and water, texture, melting properties, springiness and shredding properties. Since acid casein and sodium caseinate contain very little calcium, they are hardly capable of forming a framework and contribute little to a firm structure. They can be easily
integrated into the processed cheese and have an excellent emulsifying capacity. Adding large amounts of acid casein has adverse effects on flavour. However, even in pure imitation fresh cheese products, the typical 'caseinate flavour' is not as noticeable if sodium caseinate is added. The casein flavour can be partially masked by adding dried skimmed milk.

Rennet casein retains its original calcium content and thereby has ability to form structures, but is hydrophobic in the same way as normal cheese. If it is to be used as the protein raw material in the manufacture of imitation cheeses, rennet casein has to be converted to a paracaseinate solution, for which suitable emulsifying salts with long chain polyphosphates are required. Rennet casein can be used in imitation cheeses to achieve a long structure without impairing the flavour of the finished product. Manufacturers like to add rennet casein to products destined for toasting, which are supposed to have good renneting properties and tailing, e.g., mozzarella substitutes for pizzas.

**Vegetable fats**

The mainly used oils are those from soya, coconut, cotton seed, groundnut and sunflower. A particular vegetable fat is selected according to factors such as availability, price, resistance to oxidation and also nutritional and physiological conditions. Hydrogenated vegetable oils are less susceptible to oxidation because of lower levels of polyunsaturated fatty acids. However, oxidation only exists in the event of any separation of fat from the processed cheese-like product. Vegetable fats do not have any adverse effect on flavor, though butter fat makes a more positive contribution.

**Vegetable proteins**

Because of their completely different composition and structure, vegetable proteins can be regarded as a poor substitute for cheeses, caseins and caseinates. The most important vegetable protein isolate is the soya protein, the other sources being groundnut protein, pea and bean protein, potato protein, cotton seed protein and wheat gluten. The vegetable proteins tend to swell up when they absorb water, particularly in the presence of emulsifying salts. The consistency is pudding like and the flow properties inferior. In trials carried out using blends of cheese, casein and soya, it was only possible to use a maximum of 30% soya isolate as a proportion of the protein content of the blend.

**Examples of product development**

**Potato Starch Derivative Imitation Cheese**

Perfect amyl gel MB, a potato starch derivative can replace over 20% of casein in some cheese formulations, reducing the costs by 10% to 15% or more. When the final product contains a blend of imitation and natural cheeses, the processor can replace even more casein, further reducing costs. Sensory tests comparing a full
casein Imitation Mozzarella to one made with perfect amyl gel MB in place of 20% of casein, shows equal preference for both products in respect of flavour, texture, appearance, stretch and melt characteristics. This medium viscosity potato starch has favourable gelling and texturizing properties. It is soluble after cooking and has excellent clarity, clean taste and lower gelation temperature (Anon., 1991).

**Processed imitation mozzarella cheese**

<table>
<thead>
<tr>
<th>Dry Ingredients</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium caseinate</td>
<td>24.55</td>
</tr>
<tr>
<td>Tapioca flour</td>
<td>3.00</td>
</tr>
<tr>
<td>Salt (NaCl)</td>
<td>2.16</td>
</tr>
<tr>
<td>Adipic acid</td>
<td>0.60</td>
</tr>
<tr>
<td>Vitamins and Minerals</td>
<td>1.47</td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>0.10</td>
</tr>
<tr>
<td>Artificial cheese flavour</td>
<td>1.50</td>
</tr>
<tr>
<td>Fat-Colour Blend</td>
<td></td>
</tr>
<tr>
<td>Hydrogenated soybean oil</td>
<td>21.29</td>
</tr>
<tr>
<td>Lactylated monoglyceride</td>
<td>0.05</td>
</tr>
<tr>
<td>Red orange colouring</td>
<td>0.011</td>
</tr>
<tr>
<td>Liquid Flavour Blend</td>
<td></td>
</tr>
<tr>
<td>Various cream, cheese, starter and butter flavours</td>
<td>0.23</td>
</tr>
<tr>
<td>Water-Colour Blend</td>
<td></td>
</tr>
<tr>
<td>Colouring</td>
<td>0.05</td>
</tr>
<tr>
<td>Water</td>
<td>48.35</td>
</tr>
</tbody>
</table>

The dry ingredients are formed into a dry blend mixture by mixing them in a large Hobart mixer for 2 min. The water-colour mixture was prepared at 180°F and placed in a specially designed mixer and held at that temperature by the steam jacket on the mixer. The fat colour blend was prepared at 160°F and to this was added the liquid flavour blend. The resulting mixture was added to the hot water colour blend in the mixer and a vacuum of 20 inches of Hg was created to remove air entrapped in the contents. After about 1 min of mixing at 180°F, the fat and water emulsion was formed. The vacuum was released and the dry blended mixture added to the mixer. Vacuum was again created and held during mixing at about 170°F. After about 3 min of mixing under high shear conditions, the product, which had a pH of 5.3, was removed and packaged. After 3 days of storage at 40°F, it was sufficiently firm to shred or slice properly. This type of cheese was used for pizza and hot cheese sandwiches (US Patent No.4, 101, 413).
Comparison of the functional characteristics of commercial low moisture mozzarella cheese (LMMC) and analogue pizza cheese (APC) indicates that both cheeses have similar mean values for melt time, flowability and apparent viscosity. However, the stretchability of APC is generally inferior to that of LMMC. The differences in stretchability between LMMC and APC may be related primarily to differences in the degree of aggregation and microstructure of the paracasein caused by differences in the procedures used to manufacture the two products. During the manufacture of LMMC, the cheese curd at around pH 5.15 is subjected to a plasticization process, which involves heating to around 55 – 60°C and kneading the curd in hot (e.g., 70°C) water or dilute brine. These conditions promote a limited degree of aggregation and contraction of the paracasein gel matrix and thereby lead to the formation of paracasein fibers with a high tensile strength. The cheese fat is physically entrapped between the paracasein fibers. In contrast, the conditions used in the manufacture of APC are designed to disaggregate and hydrate the paracasein aggregates of rennet casein and caseinate. The hydrated paracaseinate immobilizes large quantities of added water and emulsifies the added vegetable oil, thereby, contributing to formation and physicochemical stability of the product. Hence, unlike LMMC, the casein in the APC is in the form of a partially hydrated dispersion rather than paracasein fibers.

**Fig. 1 Typical manufacturing procedure for analogue pizza cheese.**

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Table 1. Functionality of low-moisture mozzarella and analogue pizza cheese

<table>
<thead>
<tr>
<th>Functional attributes</th>
<th>Low-moisture mozzarella</th>
<th>Analogue pizza cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggregation index</td>
<td>3.95</td>
<td>3.74</td>
</tr>
<tr>
<td>Melt time (Sec)</td>
<td>108</td>
<td>105</td>
</tr>
<tr>
<td>Flowability (%)</td>
<td>53</td>
<td>42</td>
</tr>
<tr>
<td>Stretchability (Cm)</td>
<td>87</td>
<td>28</td>
</tr>
<tr>
<td>Apparent viscosity (Pa.s)</td>
<td>630</td>
<td>650</td>
</tr>
</tbody>
</table>

**Low fat cream cheese**

An imitation cheese product suitable for low energy diets is prepared by admixing milk, a milk fat containing carrier and dried skim milk to form a dressing mixture. It is then pasteurized and homogenized, heated and agitated at about 165-190°F, a stabilizer added and admixed with cottage cheese curd to form a curd mixture while maintaining the temperature in the range of 160 to 175°C. Preservatives and flavourings may be added prior to final homogenization. The product is hot packed and has a shelf life of 90 days with conventional refrigeration (US Patent No. 4, 724,152).

**Using concentrated milk**

Milk concentrated to 31% TS and 30% fat in conventional evaporators is pasteurized and incubated with a mixed starter at 40°C for 6 h with the pH falling from about 6.2 to 5.2. The temperature is then reduced to 24°C, another mixed starter added and incubation continued for a further 10 h with the pH falling to about 4.7. The final product is said to have exceptional aroma and body equal to excellent cheeses and lacking almost completely any free serum. By subjecting it to heating at 120-140°C, a processed type of cheese is obtained (European Patent No. 0141, 615).

**Cheese analogue from rennet and acid casein**

A paste of rennet casein is formed with an emulsifying salt solution at 110-115°F, an equal amount of acid casein is blended with the paste at the elevated temperature, until it is completely dispersed followed by the addition of edible oil (e.g., soybean oil) and other cheese analogue ingredients at 165-180°F and pH adjusted to 5.4-6.0. Mixing is continued until a homogenous, smooth plastic mass is obtained and is then packaged. The total casein content of the end product is preferably 15-30% (US Patent No. 4,397,926).

The substitution of cheddar cheese with rennet casein curd was done to formulate processed cheese preparations with reduced cost (Varghese and Sachdeva, 2002). A process for the preparation of directly acidified rennet casein curd with improved melting characteristics was standardized (Fig. 2). Blends containing 40%, 20% and 0% cheddar cheese along with the rennet casein and butter were processed using commercial emulsifying salts and synthetic flavour. No significant difference was observed between samples prepared, in terms of flavour, body & texture, sliceability.
and colour and appearance, although meltability decreased with increased content of rennet casein curd. Cost reduction to the extent of 35% was achieved in samples containing no Cheddar, without any adverse effect on sensory properties.

**Fig. 2: Procedure for the Imitation Processed Cheese preparations**
Cheese-like products from ultrafiltered retentates

The use of fresh UF skim milk retentates, the so-called total milk protein (TMP), as a major ingredient in processed cheese analogues manufacture is still in its early stages of realisation. Abou El-Nour et al. (1996) reported that up to 40% rennet casein can be replaced by TMP for obtaining fully acceptable block type processed cheese analogue. A further increase of TMP resulted in undesirable properties.

A processed cheese-like product was made from 25 lb of skim milk, 256 lb milk retentate (40% TS), 24.1 lb of two modified corn starch products, salt preservatives and colouring. Into this mixture was shredded 256 lb of Cheddar cheese before heating in a swept surface heat exchanger at 180°F for 3-5 min. WPC may also be added to the mixture (US Patent No. 4,556,569).

Use of whey solids in imitation cheese

Based on the meltability and stringiness of imitation Mozzarella cheese, Nishiya (1991) concluded that 25% of the casein could be replaced by WPC. Hsieh et al. (1993) observed that like caseinate, egg white and soya protein, whey protein also altered the viscoelastic properties of Mozzarella cheese. Soya flour (4%) dispersed in whey or soymilk mixed with whey or WPC (1: 1) could be coagulated to produce tofu. An increasing level of WPC (3-6%) added to soymilk increased the product's stiffness (Wu and Peng, 1983).

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Peanut-based Cheese

Cheese-like products from peanut milk have had limited success mainly because milk-like extracts from legumes do not produce a coagulum firm enough for making hard type cheese. Cheese analogues produced from a blend of peanut protein isolate, calcium/sodium caseinate, oil, emulsifiers and water had a consistency from firm

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type cheese to cream cheese and spread type products depending on the amount of peanut isolate used (Chen et al., 1979).

Six formulations of an imitation cheese spread were prepared by mixing cheese, flavouring, colouring, salt and dried buttermilk into a bland, light coloured groundnut paste. Three levels of flavourings (4, 6 and 8%) and two levels of salt (0.5 and 1.0 %) were used. Hedonic responses from 200 Phillipino consumers indicated that 2 formulations, i.e., 4% flavouring/1.0% salt and 6% flavouring/0.5% salt were preferred. Compared with commercial cheese spreads, the groundnut based spreads had higher protein and oil contents and lower moisture, indicating a more nutritious, potentially shelf-stable product (Santos et al., 1989).

**Conclusions**

Imitation cheese products are competing with regular cheese on an increasing scale and wider market areas. It would be unrealistic not to accept that Imitation cheese products will now offer competition for a share of the cheese market. The dairy industry has to take the view that imitation products are the result of developments in product technology and market demand. Thus not to get involved would mean curtailment of product innovation and market opportunities. Imitation cheese products can be considered an outlet for milk products and components. In developing countries, where dairy products are expensive and insufficient in quantity, dairy substitutes prepared from non-dairy proteins from legumes like soybean and peanuts, provide a nutritious alternative. By careful selection of ingredients and manufacturing conditions, different types of imitation cheese products with a lower price and lower calorific value can be manufactured. Further, attempts in coming out with new recipes of imitation cheeses and cheese analogues appear to have an unlimited scope.

**References**

• United States Patent No, 4,104,413.
• United States Patent No.4, 724,152.
• United States Patent No. 4, 39, 926.
Introduction
Cheese production is one of the oldest forms of biotechnology, dating perhaps from 6000 BC. It is one of the most diverse food groups. Cheese is the generic name for a group of fermented milk-based food products, produced in a great range of flavors and forms throughout the world. In spite of its long history, cheese still has a very vibrant image and enjoys consistent growth of about 4% per annum. It has been suggested that there are more than 1000 cheese varieties. According to the Food and Agricultural Organization of the United Nations, over 18 million metric tons of cheese was produced worldwide in 2004. Cheese is in fact one of the most scientifically interesting food groups. Although, milk clotting enzymes are relatively cheap, rennet represents the largest single industrial application of enzymes with the world market of over 150 million US dollars.

Rennet and rennet substitutes
In rennet curd cheeses, the coagulum is formed by the activity of the coagulant, an enzyme mixture with particular proteolytic activity. Most proteases will coagulate milk under suitable conditions, but most are too proteolytic related to their milk clotting activity. Consequently, they hydrolyse the coagulam too quickly, causing reduced cheese yield and/or defective cheese with a propensity to bitterness. Although plant proteinases appear to have been used as rennets since pre historic times, gastric proteinases from calves, kids or lambs have been used traditionally as rennets, with very few exceptions.

Vegetable rennet
Many plants have coagulating properties. Homer suggests in the Iliad that the Greeks used an extract of fig juice to coagulate milk (Fox et al., 2004). Other examples include nettles, thistles, mallow, and Ground Ivy (Creeping Charlie). Enzymes from thistle or cynara are used in some traditional cheese production in the Mediterranean. Phytic acid, derived from unfermented soybeans, or genetically modified (GM) soy rennet may also be used.

These real vegetable rennets are also suitable for vegetarians. Vegetable rennet might be used in the production of kosher and halal cheeses but nearly all kosher cheeses are produced with either microbial rennet or genetically modified rennet.
Worldwide, there is no industrial production for vegetable rennet. Commercial so-called vegetable rennets usually contain rennet from the mold *Mucor miehei*.

**Animal rennet**

Animal rennet is prepared by extracting the dried or salted gastric tissue with 10% NaCl and activating and standardizing the extract. Like many other proteinases, chymosin is secreted as its zymogen, prochymosin which is auto catalytically activated on acidification to pH 2-4 by removal of a 44 residue peptide from the terminal of the zymogen (Foltman, 1993).

Chymosin is well characterized at the molecular level (Foltman, 1993). The enzyme which is crystallized in the 1960's is a single chain polypeptide containing about 320 amino acid residues with a molecular mass of 35,600. Its primary structure has been established and a considerable amount of information is available on its secondary structure. Chymosin is the desired coagulating enzyme in calf rennet but because of its cost, demand and lack of calf-stomachs, most chymosin used in United States is produced by genetically engineered bacteria, yeast and molds. Fermentation derived chymosin is highly purified (100%) purity and is used in liquid and tablet form. Chymosin is the preferred coagulant because it has specificity towards one peptide bond in K-casein. Although, chymosin hydrolyses bonds in casein molecules at other sites when they are assessable, the specific site of hydrolysis that occurs during coagulation is the Phe105-Met106 of k-casein.

Like chymosin all commercially successful rennet substitutes are acid proteinases. The molecular and catalytic properties of the principal rennet substitutes are generally similar to those of chymosin (Foltman, 1993). Acid proteinases have a relatively narrow specificity, with a preference for peptide bonds to which a bulky hydrophobic residue supplies the carboxyl group. This narrow specificity is the success of these enzymes in Cheese manufacture. The fact that in cheese, these enzymes operate at a pH far different from their optima is probably also significant. However, not acid proteinases are suitable as rennets. The non specific proteolytic activity of some coagulants (not chymosin) even under the relatively unfavorable conditions causes concern over excessive proteolysis, leading to a soft body, bitter flavor defects and loss of cheese yield.

**Rennet substitutes**

**Mold rennets**

Owing to increasing world cheese production (up to 4% per annum over the past 20 years), concomitant with a reduced supply of calf rennet, the supply of this enzyme has been inadequate for many years. This has led to an increased price of calf rennet and to search for rennet substitutes. Despite the availability of numerous potentially useful milk coagulants, only six rennet substitutes have been found to be more or less acceptable for cheese production. They are bovine, porcine and chicken pepsins.
and the acid proteases from *Rizomucor mehei, R. pusillus* and *Endothia parasitica*. The proteolytic specificity of the three commonly used fungal rennets is considerably different from that of calf rennet, but the quality of most cheese varieties made using these enzymes have been fairly good.

Microbial rennets from a number of producers, e.g. Novo Nordisk, Gist- brocades, Chr. Hansen and Miles have been proved satisfactory for the production of different kinds of cheeses. They are marketed under various trade names (Rennilase, Formase, Marzyme, Hannilase). The enzyme cost for treating milk is considerably lower than using standard rennet (Burgess & Shane, 1983. The microbial rennet Rennilase, from Novo Nordisk A/S, is produced by submerged fermentation of a selected strain of the fungus *Rhizomucor miehei*. Various modifications of this enzyme have been developed- The main difference being the thermo stability of the enzyme itself. This helps makers to develop the many different types of cheeses under the local conditions (Budtz, 1989)

**Bacterial rennets**

The organisms found capable of producing the milk clotting enzymes belonging to the group of aerobic spore forming bacteria placed under the genus *Bacillus*. The enzyme has been optimally produced in a 10-liter fermenter at NDRI. The dried enzyme powder can be used as rennet substitutes in cheese manufacture. Cheddar cheese prepared with some minor technological modifications and using bacterial enzyme (*B. subtilis* K-26) has been found to be comparable to calf rennet on organoleptic evaluation.

**Table 1: Nomenclature and sources of major proteases in rennets**

<table>
<thead>
<tr>
<th>Protease</th>
<th>IUB Name &amp; Number</th>
<th>Other Names</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsin</td>
<td>Pepsin A EC 3.4.23.1</td>
<td>Pepsin II</td>
<td>Ruminants, Pigs, Chicken</td>
</tr>
<tr>
<td>Gastricsin</td>
<td>Gastricsin EC 3.4.23.3</td>
<td>Pepsin I Parapepsin II Pepsin B Pepsin C</td>
<td>Ruminants, Pigs</td>
</tr>
<tr>
<td>Chymosin</td>
<td>Chymosin EC 3.4.23.6</td>
<td>Rennin</td>
<td>Ruminants</td>
</tr>
<tr>
<td>Mucor miehei protease</td>
<td>EC 3.4.23.6</td>
<td>Rennilase (Novo) Hannilase (Chr. Hansen) Formase (Wallerstein) Marzyme (Miles)</td>
<td>Mucor miehei</td>
</tr>
<tr>
<td>M. pusillus protease</td>
<td>Emporase (Dairyland)</td>
<td>M. pusillus var. Lindt</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------------</td>
<td>-----------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meito (Meito Sangyo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Noury (Vitex)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothia parasitica protease</td>
<td>Surecurd Suparen</td>
<td>Endothia parasitica</td>
<td></td>
</tr>
</tbody>
</table>

Like chymosin, all commercially successful rennet substitutes are acid proteinases. The molecular and catalytic properties of principal rennet substitutes are generally similar to those of chymosin (Foltman, 1993). Acid proteinases have relatively narrow specificity, with a preference of peptide bonds. The milk clotting activity of the pepsins, especially porcine pepsin, is more pH dependent than that of chymosin, while that of fungal rennets is less sensitive. The coagulation of milk by *C. parasitica* protease is also less sensitive to Ca$^{++}$ than when calf rennet is used but coagulation by *Rhizomucor* proteinases is more sensitive than the rates of gel forming differ.

The thermal stability of rennets is important when the whey is to be used for processing. The early fungal rennets where considerably more thermostable than chymosin or pepsins but present products have been modified and have been modified and have thermal stability similar to that of chymosin. The thermal stability of all rennets increases with decreasing pH.

**Recombinant rennets**

Due to the storage of calf stomachs and the economic value of rennet, calf chymosin was one of the first mammalian enzymes which was cloned and expressed in microorganisms. Many different laboratories have cloned the gene for calf prochymosin in *E. coli* and analyzed the structure of the gene as well as the properties of the recombinant chymosin. The enzymic properties of the recombinant E. coli chymosin are distinguishable from those of native calf chymosin. The gene for prochymosin has also been cloned in *Sachharomyces cerevisiae*, *Kluyvermyces marxianus* var. *lactis*, *Asperillus nidulans*, *Aspergillus niger* and *Trichoderma reesei*. The cheese making properties of recombinant chymosin have been assessed on many cheese varieties, always with very satisfactory results (Teuber, 1990). These enzymes are now widely used and have taken market share from both calf rennet and especially fungal rennets. At present, attention is focused on elucidating the relationship between enzyme structure and function, but this work may lead to rennets with improved milk clotting activity or modified general proteolytic activity on $\alpha_s$- and $\beta$- casein. In 1999, about 60% of US hard cheese was made with genetically engineered chymosin and it has up to 80% of the global market share for rennet (Jhonson and Lucey, 2006). By 2008, approximately 80% to 90% of commercially made cheeses in the US and Britain were made using GM-
based rennet. The genes for *R. miehei* proteinase has been cloned in and expressed by *A. oryzae* (Novo Nordisk A/S, Denmark). It is claimed that this new rennet (Marzyme GM) is free of other proteinase/peptidase activities present in the fungal rennets and which may reduce cheese yields. Chen et al. (1996) reported excellent cheese making with Marzyme GM.

Table 2: Recombinant chymosin preparations

<table>
<thead>
<tr>
<th>Source of DNA</th>
<th>Producing organism</th>
<th>Producing Company, Brand Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf abomasums</td>
<td><em>Kluveromyces lactis</em></td>
<td>Gist Brocades; Maxiren</td>
</tr>
<tr>
<td>Calf abomasums</td>
<td><em>Aspergillus niger</em></td>
<td>Genecor/ Chr. Hansen; Chymogen</td>
</tr>
<tr>
<td>Synthetic</td>
<td><em>Escherichia coli</em></td>
<td>Pfizer; Chy Max</td>
</tr>
</tbody>
</table>

Immobile rennets

More than 90% of the rennet activity added to cheese milk is lost in the whey, representing an economic loss and creating problems for whey processors. Both the problems could be resolved if immobilized rennets could be used to coagulate milk. Another incentive for the immobilization of rennets is the possibility of producing cheese curd continuously. Since a small proportion, of chymosin or rennet substitutes is retained in cheese curd, it plays a major role in cheese ripening and, therefore, if immobilized rennets were used successfully to coagulate milk, it would be necessary to add rennet or other proteinase to the curd. But again uniform incorporation of this enzyme would be problematic.

Academically, the availability of completely immobilized, effective rennet would permit the manufacture of rennet-free curd for studies on the contribution of enzymes from different sources to cheese ripening. A number of approaches have been used to produce rennet-free curd (Fox et al., 1993).

The first report on immobilized rennet is that of Green and Crutchfield (1969) who successfully immobilized the enzyme but found that leaching occurred and the free enzyme coagulated the milk. This was followed by a series of publications, who concluded that milk could be coagulated by immobilized rennets (Taylor, 1976). However, Fox (1981) concluded that the published data did not confirm with the known kinetics and characteristics of the rennet coagulation of milk and suggested that coagulation was caused by solubilized rennets. Carlson et al. (1986) and Dalgleish (1987, 1995) also reached similar conclusions. But still publications on the coagulation of milk by immobilized rennets continue to appear e.g. Shah et al. (1995). The dissociation of proteinases from support is probably the main reason why clotting has been observed in many studies. At normal temperatures and pH values, extensive proteolysis of the k-casein is required before the micelles will aggregate and so it is likely that any experiments where coagulation occurs at low extents of proteolysis, and at normal temperature and pH must be doubted. This is confirmed by the observation that apparently fully immobilized chymosin hydrolyzed only limited amounts of k-casein and no clotting was observed.
Therefore, although it is in principle possible to achieve clotting via very careful use of reactors involving immobilized enzymes, the general opinion must be that this cannot be practicable in anything larger than a laboratory experiment.

References

- Teuber, M. (1990) Production of chymosin (E.c. 3.4.23.4) by microorganisms and its use for cheese making. IDF Bulletin, 251: 3-15
RHEOLOGICAL PROPERTIES OF CHEESE

Prateek Sharma, Richa Badola and Dr. A.A. Patel

Introduction

The term Rheology was coined by Professor E.C. Bingham to represent a branch of mechanics concerned with the study of deformation and flow of materials when subjected to a stress or strain. However, the term texture and rheology are closely linked with each other especially in relation to products like cheese. The texture is defined as an external manifestation of internal structural arrangements of macromolecules, generally perceived by human auditory and tactile senses, and sometime by instrumental measurement viz. texture profile analysis (TPA).

The rheological properties of cheese are of considerable importance, since they affect its handling, portioning, and packing characteristics, texture and eating quality, uses as an ingredient, ability to retain a given shape at a given temperature or when stacked, ability to retain gas and hence to form eyes or cracks or to swell etc. The present discussion, therefore, emphasized on rheological properties of cheese and factors affecting them and also methods/approaches to measure rheological attributes of different cheese varieties.

Methods of rheological measurements on cheese

Rheological measurements may be categorized into three regimes for viscoelastic solid foods (Fig. 1). The first region is the linear realm, where the relationship between stress and strain is proportionate, and Hooke’s law is obeyed. The next region is one of nonlinearity; the nonlinear regime is as the name implies a more complex relationship between stress and strain. Finally, the last point occurs at sample fracture. Regions I and III have been well characterized for food materials due to the simplicity of gathering and interpreting measurements in these zones. For example, small amplitude oscillatory shear can only measure rheological properties in region I, and testing to failure (point III) can be easily quantified and explained. However, to describe the sensory response during mastication of food products, region II cannot be ignored and may profoundly and significantly contribute to the understanding of texture analysis and sensory description.

Texture measurement techniques can be grouped as either subjective or instrumental. The subjective measurements or sensory evaluation are made by the trained taste panel. The instrumental methods can be broadly grouped under the following three categories: empirical, imitative, and fundamental.
Empirical tests involve subjecting a cheese sample to a stress or strain by various techniques (e.g., inserting a penetrometer). The different types include: Compression tests, where the extent of compression under a constant load for a

Fig. 1 Rheological regimes for viscoelastic solid foods

Empirical methods are based on the subjection of cheese to a stress or strain that results in visual fracture i.e. permanent deformation. In these tests, the test conditions are arbitrary and the aim is to obtain a number that gives a vague indication of the textural characteristics of the cheese (e.g., its hardness). Empirical tests are relatively simple procedures that typically measure a force on a sample and the accompanying deflection. These methods rely completely on test parameters, such as sample volume, shape, and testing speed. The penetrometer, puncture test, and ball-compressor tests are good examples of empirical measurements.

Imitative methods which may also be called semifundamental methods include measurement systems that are used to make mechanical measurements with little control of experimental variables (e.g., probe type and size, product shape, etc.). They attempt to mechanically mimic the sensory evaluation of human evaluators. In fact, when the instrumental test used mimics the action of the human assessor, more accurate models of food texture attributes could be developed. The test results from imitative tests are analyzed and correlated to sensory perceptions of taste panels without valid structural and molecular-level reasoning. Therefore, the test results, at best, serve as relative measures of textural attributes of products tested. Nonetheless, the imitative methods are perhaps the largest group of instrumented texture-measurement methods. The widely adopted texture profile analysis (TPA) belongs to this group.

Fundamental methods employ valid rheological test techniques, and the data are analyzed using well-defined rheological, structural, and molecular theories. The fundamental test methods also yield results that are independent of test instrument.

Also some non-destructive methods have also being developed to measure rheology in cheese.

Empirical tests

These tests involve subjecting a cheese sample to a stress or strain by various techniques (e.g., inserting a penetrometer). The different types include: Compression tests, where the extent of compression under a constant load for a
specified time is measured (e.g., ball compressor test); Penetration tests, where the force required to insert a probe a given distance into the cheese or, alternatively, the depth of penetration by a probe under a fixed load for a given time is measured (e.g., penetrometer test); Cutting tests, measure the resistance to the passage of a knife or a wire through a cheese (e.g., Cherry-Burell Curd tension meter); Curd tests, measures the characteristics of the curd. Various tests come under this which measures firmness, pitching point etc. of the curd.

**Ball compressor test**

The ball compressor test measures the depth of indentation after a given time made by a small ball or hemisphere when placed under a given load (stress) on the cheese surface (Fig. 2). The depth of penetration has been used directly as an index of firmness. Alternatively, by making a number of simple assumptions, testers may use it to calculate a modulus, analogous to an elastic modulus (\(G\), given by the equation

\[
G = 3M[16(RD^4)]^{1/2}
\]

where, \(M\) is the applied force and \(R\) and \(D\) are the radius and depth of the indentation, respectively).

![Fig. 2 The ball compressor (diagrammatic)](image)

The Ball Compressor has the merits of cheapness and simplicity, but the time taken to obtain a representative reading limits its use to the research laboratory.

**Penetrometer test**

The penetrometer test is one other empirical test, which is known for its simplicity and not quite nondestructive, but very nearly so, since it only requires that a needle be driven into the body of the cheese; no separate sampling is required. It measures the depth to which a penetrometer (e.g., needle or cone or rod) can be forced into a
cheese under a constant stress. As the needle or cone penetrates the cheese, the cheese in its path is fractured and forced apart. The progress of the penetrometer is retarded to an extent that depends on the hardness of the cheese in its path, the adhesion of the cheese to its surface (which increases with the depth of penetration into the cheese), and its surface area of contact with the cheese (regulated by the thickness of the needle or angle of the cone used). Eventually, the retardation stresses become equal to the applied stress and penetration ceases. In case of cone penetrometer (Fig. 3), the penetration depth at rest \( h \) is used to calculate an “apparent yield stress,” \( \sigma_{\text{app}} \):

\[
\sigma_{\text{app}} = \frac{M g}{\pi h^2 \tan^2(\alpha/2)}
\]

where, \( M \) is the cone mass, \( g \) the acceleration due to gravity, and \( \alpha \) the cone angle. For a given cone of known mass and cone angle, the equation simplifies in terms of just \( h \), i.e., \( \sigma_{\text{app}} = k/h^2 \), where, \( k \) is a constant for the particular cone. Cone probes with various angles (e.g., 20–90°) are available to be used with commercial instruments. This test, which is used to provide an index of hardness (i.e., resistance of a surface to penetration) is suitable for closed-textured cheeses such as Gouda and Mozzarella, which are macroscopically homogeneous. Conversely, it is unsuitable for open-textured cheeses with small mechanical openings or eyes (e.g., Tilsit and Gruyere) or cheeses that are macroscopically non-uniform owing to the presence of chip boundaries (e.g., Cheddar).

**Fig. 3 Illustration of a constant-weight cone penetrometer test**

\( H \) = cone height;
\( \alpha \) = cone angle, \( h \) = cone penetration depth

**Cutting test**

A sectilometer consists of a tightly drawn wire which is made to cut through the sample blocks of specified dimensions, and the force required for the wire to cut through at a constant rate is recorded. Recently, a programmable version of the sectilometer called ‘Buttomat’ (Dinkelberg, Germany) has been developed.
Curd test

During the early part of cheese making, curd formation is characterized by increase in viscosity as a result of curd structure development. There are various empirical tests developed to monitor curd characteristics, some of them are based on a rotating or oscillating drum, or an oscillating blade used to monitor curd firmness, where the torque is due initially to the viscous drag of the renneted milk and later to the rigidity of the curd. The Thromboballastograph or Lactodynamograph, essentially a torsiometer, working on this principle, has been extensively used to determine the rennetability of milk. Other such instruments include the one which entail generating a pressure pulse through the curd whose transmission depends on the curd rigidity. Some of the devices have also been used for continuous monitoring of the curd firmness during setting. The pitching point apparatus meant for measuring pitching number expressing the bulk density as related to the stand-up for cheese rheology.

Imitative tests

These tests involve use of instrument that mechanically mimic the action of the human and thus being more accurate. The most common example of imitative test is Texture profile analysis. Nonetheless, the imitative methods are perhaps the largest group of instrumented texture-measurement methods. The widely adopted texture profile analysis (TPA) belongs to this group.

Texture profile analysis (TPA)

Current TPA test is essentially a uniaxial compression test. A system of rheological parameters (e.g., firmness, elasticity) related to texture and known as TPA was developed. Texture profile analysis parameters were later calculated from measurements using uniaxial doublebite compression at constant speed, using texture analysers including the Instron UTM and the texture analyser (TA series from Stable Micro Systems)(Fig. 4.).

![Fig. 4 Typical stress trend during a double-bite compression test, from which TPA parameters are calculated (see Table 1)]
Table 1 Texture profile analysis (TPA) parameters and physical definitions

<table>
<thead>
<tr>
<th>Terminology</th>
<th>Physical definition (TPA term)</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fracturability</td>
<td>Stress (or sometimes, force) to fracture point, H1</td>
<td>Pa, kPa</td>
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<tr>
<td>Firmness</td>
<td>Stress (or sometimes, force) at a given deformation</td>
<td>Pa or kPa</td>
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<tr>
<td>Springiness (or elasticity)</td>
<td>Percentage of deformation which is recovered between the first and second bites</td>
<td>–</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>Area of second bite over area of the first bite (A2/A1)</td>
<td>–</td>
</tr>
<tr>
<td>Gumminess</td>
<td>Hardness X Cohesiveness</td>
<td>Pa, kPa</td>
</tr>
<tr>
<td>Chewiness</td>
<td>Hardness X Cohesiveness X Springiness</td>
<td>Pa, kPa</td>
</tr>
<tr>
<td>Adhesiveness</td>
<td>Work necessary to pull the plunger (or compression plate) away from the sample (Area 3)</td>
<td>J/m³</td>
</tr>
</tbody>
</table>

Fundamental tests

These tests which are used to measure rheological measurements on cheese can be further grouped as:-

**Static methods**

These are the methods in which the sample is stressed constantly in the same direction and corresponding strain or deformation is measured. The stress-strain curve obtained is characteristic of the sample. A very good example of this is force-compression tests which measures the rheological behavior of cheese as the strain is increased over time to values that generally result in fracture and flow of the cheese being tested. The tests, which are today commonplace for the measurement of fracture properties of cheese.

**Transient methods**

During transient tests an instantaneous and constant load or deformation is applied to the material, and the responding behavior is monitored with the lapsing time. For example, a creep test places an instantaneous and constant stress on the material while the strain or compliance (J) is measured with time (Fig. 5). Data from creep tests may determine retardation time constants (λ) of the sample, a characteristic time descriptor for the material. Similarly, an instantaneous strain may be applied, and the decay of the stress may be recorded with time and stress relaxation time is obtained to characterize viscoelastic product. These time constants (relaxation and retardation time), provide insight as to the ease a material can adapt to an applied load and the larger the constant, the slower material relaxation. A second component to a creep test is recovery upon load removal. If the sample displays no recovery, maintaining a constant degree of strain or compliance, the sample has a significant viscous component.
Dynamic methods

In dynamic tests material is subjected to sinusoidally varying stresses or strains while recording the rheological response (Fig.6). To determine viscoelastic properties with dynamic tests, samples must be measured within the linear viscoelastic region, region I of Fig. 2. During the oscillatory test, the sample either stores energy or dissipates energy in the form of heat. A purely elastic substance will store all energy, while an ideally viscous material will dissipate and lose the energy. The ratio of energy lost to energy stored is an important parameter known as the phase angle (δ) and describes the relative degree of viscoelasticity. For example, a purely elastic material will have a phase angle of 0°. Since Hooke’s Law, a proportional region between stress and strain, is obeyed for these tests, the ratio of stress and strain amplitudes (σ₀; γ₀) provides another important viscoelastic property, the complex modulus (G*). This modulus, in conjunction with the phase angle, can produce the two primary viscoelastic terms of interest, the storage (G’) and loss (G”) moduli:

\[
\begin{align*}
G' &= G^* \cos (\delta), \\
G'' &= G^* \sin (\delta)
\end{align*}
\]

The storage modulus reflects the degree in which a material stores energy (elastic component), while the loss modulus describes the degree of dissipation (viscous component). These two viscoelastic properties are typically measured while the oscillatory frequency is changing to produce the mechanical spectra of the sample, or a rheological fingerprint of the small strain behavior for the material.
Traditionally, cheese texture has been evaluated by destructive sensory and instrumental measurements. TPA, uniaxial compression and puncture tests have been widely used to assess cheese texture, providing information on both the deformation and fracture properties of food products.

**Acoustic impulse–response technique**

The acoustic impulse–response technique is a fast, nondestructive measurement of firmness where the food is excited by being struck with a probe and the frequency spectrum from the recorded sound is obtained. The set-up consists of the same elements used for the force–deformation experiments and additionally a microphone with pre-amplifier (Fig. 7). The signal conditioner worked at 20 dB gain and 10 Hz high pass filter. Pieces of cheese sample are excited by the probe which struck on the cheese surface. The sound generated by each impact was acquired by
the microphone and digitized at 20 k samples/s. The Fast Fourier Transform (FFT) of the signal is calculated using Excel™ and the resulting spectrum analyzed in order to obtain information on the different modes of vibration of the cheese samples. The microphone is placed 2 cm above the flat cheese surface and 5 cm from the center of the pieces. Ultrasound waves being more sensitivity are preferred for the nondestructive characterization of texture of the cheese.

**Rheological properties of cheeses**

The diversity of cheese types is truly breathtaking but what is common among them, is their viscoelastic or plasto-viscoelastic nature (Table 2). The rheological properties of cheese have a large influence on its texture and behaviour during size reduction, and hence, its suitability as an ingredient. Many factors influence the rheological properties, including manufacturing procedure, variety, composition and biochemical changes during ripening. Being complex in nature and also nonhomogeneous, the various instrumental methods such as texture profile analysis, uniaxial compression, puncture, and cutting test which are used to measure the rheological properties of cheeses cannot be wholly relied for accuracy. However, briefly rheologies of some of the varieties of cheeses are being discussed below:

**Natural cheeses**

Earlier cheese varieties were compared on the basis of their apparent viscosity, shear modulus etc. For example various English cheeses including Cheddar (viscosity, $1.38 \times 10^{10}$ Pa.s; shear modulus, $1.13 \times 10^{8}$ Pa) could be differentiated in terms of consistency, which matched the sensory classification by expert graders. However, in the recent times, importance have being given to hardness (peak force) which is measured using compression-based TP analysis, and found relating to the sensory consistency of Cheddar and other cheeses. Some studies also suggest the significance of degree of compression which has a considerable bearing on hardness value, because it has been found that the harder cheese fracture at lower levels of compression as compared to softer cheeses.

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<th>Rheological property</th>
<th>Definition</th>
<th>Cheese type displaying property</th>
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</thead>
<tbody>
<tr>
<td>Elasticity (rubberiness)</td>
<td>Tendency of cheese to recover its original shape and dimensions upon removal of an applied stress</td>
<td>Swiss-type cheese, low-moisture Mozzarella</td>
</tr>
<tr>
<td>Springiness</td>
<td>Tendency to recover from large deformation (strain) after removal of deforming stress</td>
<td>Swiss-type cheese, low-moisture Mozzarella</td>
</tr>
<tr>
<td>Elastic Fracturability</td>
<td>Tendency of hard cheese to crack, with very limited flow (confined to vicinity of crack); after fracture, the broken surfaces can be fitted to each other</td>
<td>Parmesan, Romano, Gruyere</td>
</tr>
<tr>
<td>Brittleness</td>
<td>Tendency of hard cheese to fracture at a</td>
<td>Romano, Parmesan</td>
</tr>
</tbody>
</table>

Table 2 Rheological properties of cheeses and their definitions
Firmness (hardness) | High resistance to deformation by applied stress | Cheddar, Swiss-type cheese, Romano, Parmesan, Gouda
Longness | The resistance of cheese to fracture until a relatively large deformation is attained | Mozzarella, Swiss
Toughness (chewiness) | A high resistance to breakdown upon mastication | Mozzarella, String cheese, Halloumi
Softness | Low resistance to deformation by applied force | Blue cheese, Brie, Cream cheese
Plastic Fracturability | The tendency of cheese to flow on fracture | Mature Cheddar, Blue cheese, Chaumes, Raclette
Shortness | The tendency to plastic fracture at a small deformation; low resistance to breakdown upon mastication | Camembert, Brie
Adhesiveness (stickiness) | The tendency to resist separation from another material with which it makes contact (e.g., another ingredient or a surface such as a knife blade or palate) | Mature Camembert
Crumbliness | The tendency to break down easily into small, irregularly shaped particles (e.g., by rubbing) | Cheshire, Wensleydale, Blue cheese, Stilton, Feta
Shear thickening | The tendency to increase in apparent viscosity when subjected to an increasing shear rate (especially upon heating) | Cream cheese (when heated), 'creaming' of processed cheese products
Shear thinning | The tendency to exhibit a decrease in apparent viscosity (especially at low when subjected to an increasing shear rate temperatures, i.e., <4 °C) | Quarg

**Processed cheeses**

Sliceable processed cheese analysis through compression tests in several laboratories have exhibited yield or rupture stress values in the range of 0.68 to 1.56 \(x 10^5\) N/m² and an apparent shear modulus of 2.27 to 10.00 \(x 10^5\) N/m², whereas the corresponding values for spreadable processed cheese were 0.15 to 0.53 \(x 10^5\) N/m² and 0.99 to 3.93 \(x 10^5\) N/m², respectively.

**Semi and semi-soft cheeses**

In India, Channa and Chakka are products which belongs to the group of soft cheese, whereas Paneer and Mozzarella cheese belongs to semi-soft group. Compression methods, TPA in particular, and/or penetration have been successfully used for textural characterization of Channa as well as Paneer, but Mozzarella rheology has been investigated in far greater details.
Conclusion

Cheese rheology, has been studied and reviewed extensively which can be attributed to the fact that the rheological properties of cheese are quality attributes that are important to the manufacturer, packager, distributor, retailer, industrial user as well as the consumer. Cheese is a visco-elastic product and its texture is subjected to changes not only during manufacturing but also during storage. Wide arrays of rheological methods have been devised to measure the rheology of cheese. However, being very nonhomogeneous in nature, considerable difficulties are faced in precise and reproducible measurements that can be compared. This makes it imperative that the test conditions for rheological measurements be carefully controlled and reported along with test results. Also most of the rheological methods are destructive in nature so there is a need to find more non-destructive techniques.

References

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<td>Dr. S. Singh</td>
<td>Ex- Joint Director (Academics) National Dairy Research Institute, Karnal</td>
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<td>Dr. G. R. Patil</td>
<td>Joint Director (Academics) &amp; Principal Scientist Dairy Technology Division National Dairy Research Institute, Karnal</td>
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<td>Dr. V. K. Batish</td>
<td>Emeritus Scientist Dairy Microbiology Division National Dairy Research Institute, Karnal</td>
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<td>Dr. Sanjeev Kumar</td>
<td>Assistant Professor Sanjay Gandhi Institute of Dairy Technology, Patna</td>
</tr>
<tr>
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<td>Mr. Yogesh Khetra</td>
<td>Scientist Dairy Technology Division National Dairy Research Institute, Karnal</td>
</tr>
<tr>
<td>Developments in Quarg cheese technology</td>
<td>Dr. S. K. Kanawjia</td>
<td>Principal Scientist Dairy Technology Division National Dairy Research Institute, Karnal</td>
</tr>
<tr>
<td></td>
<td>Mr. Kunal Kumar</td>
<td>Assistant Professor Dairy Technology Department SMC College of Dairy Science A.A.U. - Anand</td>
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<td></td>
<td>Mr. Yogesh Khetra</td>
<td>Scientist Dairy Technology Division National Dairy Research Institute, Karnal</td>
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<td></td>
<td>Mr. Alok Chatterjee</td>
<td>Research Scholar Dairy Technology Division National Dairy Research Institute, Karnal</td>
</tr>
<tr>
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<td>Dr. S. K. Kanawjia</td>
<td>Principal Scientist Dairy Technology Division National Dairy Research Institute, Karnal</td>
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<td></td>
<td>Mr. Yogesh Khetra</td>
<td>Scientist Dairy Technology Division National Dairy Research Institute, Karnal</td>
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<td>Research Scholar Dairy Technology Division National Dairy Research Institute, Karnal</td>
</tr>
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<td>Principal Scientist Dairy Technology Division National Dairy Research Institute, Karnal</td>
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<td>Dr. P. Narender Raju</td>
<td>Scientist Dairy Technology Division National Dairy Research Institute, Karnal</td>
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<td>Scientist Dairy Technology Division National Dairy Research Institute, Karnal</td>
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<td>Mr. Alok Chatterjee</td>
<td>Research Scholar Dairy Technology Division National Dairy Research Institute, Karnal</td>
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<td>Ex – Principal Scientist</td>
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<td>National Dairy Research Institute, Karnal</td>
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<tr>
<td></td>
<td>Dr. S. Makhal</td>
<td>Senior Manager</td>
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<tr>
<td></td>
<td>Mr. Alok Chatterjee</td>
<td>Research Scholar</td>
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<tr>
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<td>National Dairy Research Institute, Karnal</td>
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<tr>
<td>Popular milk based fermented products</td>
<td>Dr. Satish Kulkarni</td>
<td>Head and Principal Scientist</td>
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<td></td>
<td></td>
<td>National Dairy Research Institute, Karnal</td>
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<td>Dr. A. K. Dodeja</td>
<td>Head and Principal Scientist</td>
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<td></td>
<td>National Dairy Research Institute, Karnal</td>
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<tr>
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<td>Dr. Rameshwar Singh</td>
<td>Head and Principal Scientist</td>
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<td></td>
<td></td>
<td>National Dairy Research Institute, Karnal</td>
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<tr>
<td></td>
<td>Dr. Surajit Mandal</td>
<td>Scientist</td>
</tr>
<tr>
<td></td>
<td>Mr. R. P. Singh</td>
<td>Technical Officer 7-8</td>
</tr>
<tr>
<td>Additives in cheese making</td>
<td>Dr. A. J. Pandya</td>
<td>Head</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SMC College of Dairy Science</td>
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<td></td>
<td></td>
<td>A.A.U – Anand</td>
</tr>
<tr>
<td>Cheese-making equipment and mechanization</td>
<td>Dr. G. S. Rajorhia</td>
<td>Principal Scientist (Retd.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>National Dairy Research Institute, Karnal</td>
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<tr>
<td></td>
<td>Prof. I. K. Sawhney</td>
<td>Principal Scientist</td>
</tr>
<tr>
<td></td>
<td>Mr. P. S. Minz</td>
<td>Scientist</td>
</tr>
<tr>
<td>Food safety as applied to fermented food products</td>
<td>Dr. (Ms) Bimlesh Mann</td>
<td>Principal Scientist</td>
</tr>
<tr>
<td></td>
<td></td>
<td>National Dairy Research Institute, Karnal</td>
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<td>Fermenters and downstream processing</td>
<td>Dr. R. K. Malik</td>
<td>Principal Scientist</td>
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<td>Mr. Santosh Kumar Mishra</td>
<td>Research Scholar</td>
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<td>Dr. Raman Seth</td>
<td>Principal Scientist</td>
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<td>Ms. Anamika Das</td>
<td>Research Scholar</td>
</tr>
<tr>
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<td>Dr. (Ms) Sunita Grover</td>
<td>Principal Scientist</td>
</tr>
<tr>
<td></td>
<td>Harsh Panwar</td>
<td>Research Scholar</td>
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<tr>
<td></td>
<td>Aparna S. V.</td>
<td>M. Tech.</td>
</tr>
<tr>
<td></td>
<td>Ritu Chauhan</td>
<td>M. Tech.</td>
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<tr>
<td></td>
<td>Rashmi H. M.</td>
<td>Research Scholar</td>
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<td>Dr. V. K. Batishe</td>
<td>Emeritus Scientist</td>
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<td>Dr. Kaushik Khamrui</td>
<td>Senior Scientist</td>
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<td>Senior Scientist</td>
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<td>Dr. (Ms) Falguni Patra</td>
<td>Research Associate</td>
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<td>Dr. V. K. Gupta</td>
<td>Principal Scientist</td>
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<td>Dr. R.K. Malik</td>
<td>Principal Scientist</td>
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<td>Milk coagulating enzymes in cheese manufacture</td>
<td>Ms. Gurpreet Kaur</td>
<td>Research Associate</td>
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<tr>
<td>Rheological properties of cheese</td>
<td>Mr. Prateek Sharma</td>
<td>Scientist</td>
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<tr>
<td>Developments in fermented dairy analogues</td>
<td>Dr. A. A. Patel</td>
<td>Head and Principal Scientist</td>
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<td>Practical/Visit</td>
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<tr>
<td>Manufacture of Mozzarella cheese</td>
<td>Dr. D. K. Thompkinson (Principal Scientist)</td>
<td>Dairy Technology Division</td>
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<tr>
<td>Manufacture of processed cheese</td>
<td>Mr. Ram Swarup (Technical Officer – 6)</td>
<td>National Dairy Research Institute, Karnal</td>
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<tr>
<td>Developments in Dutch varieties of cheeses: Manufacture of Gouda cheese</td>
<td>Dr. (Ms) Latha Sabikhi, Mr. S. K. Kharb (Senior Scientist, Technical Officer - 7)</td>
<td>Dairy Technology Division</td>
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<tr>
<td>Renneting behavior of milk and curd forming characteristics</td>
<td>Dr. B. D. Tiwari (Ex- Principal Scientist)</td>
<td>Dairy Technology Division</td>
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<tr>
<td>Manufacture of milk cereal based fermented product</td>
<td>Dr. A. K. Singh (Senior Scientist), Mr. G. S. Meena (Scientist)</td>
<td>National Dairy Research Institute, Karnal</td>
</tr>
<tr>
<td>Rheological properties of cheese</td>
<td>Mr. Prateek Sharma, Mr. G. S. Meena (Senior Scientist, Scientist)</td>
<td>National Dairy Research Institute, Karnal</td>
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<tr>
<td>Electron microscopy in textural studies in bio-processed foods</td>
<td>Dr. S. K. Tomar (Principal Scientist), Mr. Ram Swarup (Technical Officer – 6)</td>
<td>National Dairy Research Institute, Karnal</td>
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<tr>
<td>Preparation of lassi/ fruit lassi</td>
<td>Mr. Yogesh Khetra, Mr. Devraja H. C. (Scientist)</td>
<td>Dairy Technology Division</td>
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<tr>
<td>Preparation of cheese spreads</td>
<td>Dr. D. K. Thompkinson, Mr. S. K. Kharb (Principal Scientist, Technical Officer – 7)</td>
<td>National Dairy Research Institute, Karnal</td>
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<td>Manufacture of Quarg Cheese</td>
<td>Dr. S. K. Kanawjia, Mr. Yogesh Khetra (Principal Scientist, Scientist)</td>
<td>National Dairy Research Institute, Karnal</td>
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<tr>
<td>Preparation of pizza</td>
<td>Mr. Yogesh Khetra, Mr. Ram Swarup (Scientist, Technical Officer – 6)</td>
<td>National Dairy Research Institute, Karnal</td>
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**Visits**

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<th>Visit</th>
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<tr>
<td>Agricultural Technology Information Centre (ATIC)</td>
<td>Dr. D. S. Sohi (Principal Scientist &amp; Incharge, ATIC)</td>
<td>National Dairy Research Institute, Karnal</td>
</tr>
<tr>
<td>National library of dairying</td>
<td>Dr. B. R. Yadav (Principal Scientist)</td>
<td>Dairy Cattle Breeding Division &amp; Head, Library Services</td>
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<tr>
<td>Model dairy plant</td>
<td>Mr. G. C. Mutreja (General Manager)</td>
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</table>
LIST OF PARTICIPANTS

ADVANCED COURSE IN FACULTY TRAINING IN DAIRY PROCESSING ON TECHNOLOGICAL ADVANCES IN CHEESE AND FERMENTED DAIRY FOODS (July 5-25, 2011)

1. Dr. Ashwani Kumar
   Assistant Professor
   Department of Biotechnology
   Seth Jai Parkash Mukund Lal Institute of Engineering and Technology
   Radaur- 135133
   Yamuna Nagar, Haryana

2. Mr. Andhare Balasaheb Chhaganrao
   Assistant Professor
   College of Agriculture, Badnapur,
   Jalna- 431201 (Maharashtra)

3. Dr. B.K. Sakhale
   Assistant Professor
   Food Technology Division
   Department of Chemical Technology
   Dr. Babasaheb Ambedkar Marathwara University
   Aurangabad – 431004
   Maharashtra

4. Dr. Bahadur Singh Hathan
   Assistant Professor,
   Department of Food Engineering and Technology,
   SLIET, Longowal,
   Sangrur (Punjab) - 148106

5. Dr. B.M. Thombre
   Associate Professor
   Deptt. of Animal Husbandry and Dairy Science,
   College of Agriculture
   Latur- 412 513 (Maharashtra)

6. Dr. Dhiraj Hiramn Kankhare
   Assistant Professor
   Division of Animal Science and Dairy Science
   College of Agriculture,
   Dhule- 424004 (MS)

7. Mr. Gokhale Ajay Jayantiao
   Assistant Professor,
   Department of Dairy Processing and Operations,
   SMC College of Dairy Science
   A.A.U., Anand- 388110

8. Mr. John David
   Assistant Professor, Dairy Technology
   Warner School of Food & Dairy Technology,
   SHIATS,
   Allahabad- 211007 (UP)

9. Mr. Kadiya Kunal Kumar
   Shashikant
   Assistant Professor,
   Dairy Technology Department,
   SMC College of Dairy Science
   A.A.U., Anand- 388110

10. Mr. Nage Sanjiv Pandurang
    Assistant Professor
    Deptt. of Animal Husbandry and Dairying
    Dr. P.D.K.V., Akola
<table>
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<tr>
<th></th>
<th>Name</th>
<th>Academic Position and Responsibilities</th>
<th>Institutional Details</th>
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<tbody>
<tr>
<td>11</td>
<td>Dr. P. K. Bhardwaj</td>
<td>Associate Professor of LPT</td>
<td>LLRUVAS Hisar</td>
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<tr>
<td>12</td>
<td>Dr. Ravinder Nagpal</td>
<td>Assistant Professor of Biotechnology</td>
<td>Seth Jai Parkash Mukund Lal Institute of Engineering and Technology</td>
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<td>Radaur- 135133</td>
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<td>Yamuna Nagar, Haryana</td>
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<tr>
<td>13</td>
<td>Dr. R.B. Sharma</td>
<td>Senior Scientist (LPT), NFR &amp; PT Division</td>
<td>CIRG, Makhdoom, Farah, Mathura - 281122 (UP)</td>
</tr>
<tr>
<td>14</td>
<td>Mr. Sandeep G. M. Prasad</td>
<td>Assistant Professor, Dairy Technology</td>
<td>Warner School of Food &amp; Dairy Technology, SHIATS, Allahabad - 211007 (UP)</td>
</tr>
<tr>
<td>15</td>
<td>Dr. Sandip Kumar</td>
<td>Subject Matter Specialist - Livestock Production and Management</td>
<td>Krishi Vigyan Kendra, Kalyanpur, Shahdol (M. P.) - 484001</td>
</tr>
<tr>
<td>16</td>
<td>Dr. Sanjeev Kumar</td>
<td>Assistant Professor of Dairy Science and Technology</td>
<td>BVC Campus, Jagdeopath, Patna-14 (Bihar)</td>
</tr>
<tr>
<td>17</td>
<td>Dr. Sambhaji Dattatraya Nalkar</td>
<td>Assistant Professor of Agricultural Biotechnology</td>
<td>College of Agricultural Biotechnology, Loni, Tal-Rahata, Dist. Ahmednagar (Maharashtra)</td>
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<td>18</td>
<td>Prof. Somnath Hanumant Mane</td>
<td>Assistant Professor of Animal Science and Dairy Science</td>
<td>College of Agriculture, Pune (MS) - 411003</td>
</tr>
<tr>
<td>19</td>
<td>Mr. Suryamani Kumar</td>
<td>Assistant Professor of Dairy Technology</td>
<td>S. G. Institute of Dairy Science and Technology, BVC Campus, Jagdeopath, Patna-14</td>
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<td>20</td>
<td>Dr. Vikas Nanda</td>
<td>Associate Professor, Department of Food Engineering and Technology, SLIET, Longowal</td>
<td>Sangrur (Punjab) - 148106</td>
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Dr. D. Sundaresan Auditorium

NATIONAL DAIRY RESEARCH INSTITUTE
(DEEMED UNIVERSITY)
(ICAR), KARNAL - 132 001 (HARYANA) INDIA
Tel. : 0184-2252800, Fax : 0184-2250042
Website : www.ndri.res.in
E-mail : dir@ndri.res.in