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Fermented Milks and Milk Products as Functional Foods—A Review

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Fermented foods and beverages possess various nutritional and therapeutic properties. Lactic acid bacteria (LAB) play a major role in determining the positive health effects of fermented milks and related products. The L. acidophilus and Bifidobacteria spp are known for their use in probiotic dairy foods. Cultured products sold with any claim of health benefits should meet the criteria of suggested minimum number of more than $10^6$ cfu/g at the time of consumption. Yoghurt is redefined as a probiotic carrier food. Several food powders like yoghurt powder and curd (dahi) powder are manufactured taking into consideration the number of organisms surviving in the product after drying. Such foods, beverages and powders are highly acceptable to consumers because of their flavor and aroma and high nutritive value. Antitumor activity is associated with the cell wall of starter bacteria and so the activity remains even after drying. Other health benefits of fermented milks include prevention of gastrointestinal infections, reduction of serum cholesterol levels and antimutagenic activity. The fermented products are recommended for consumption by lactose intolerant individuals and patients suffering from atherosclerosis. The formulation of fermented dietetic preparations and special products is an expanding research area. The health benefits, the technology of production of fermented milks and the kinetics of lactic acid fermentation in dairy products are reviewed here.

Keywords Fermented milks, L. acidophilus, bifidobacteria, probiotics, lactic acid bacteria

INTRODUCTION

Healthiness is one of the most frequently mentioned reasons behind food choices in EU countries (Lappalainen et al., 1998). Individuals are considered responsible for their own state of health. Diseases are seen more and more as consequences of consumer’s own behavior rather than the result of environment (Ogden, 1998). Diet may strongly influence an individual’s risk of obesity; cardiovascular diseases, cancer, and other life style related diseases. Traditionally, the healthfulness has been associated with nutritional factors such as fat, fiber, salt, and vitamin contents of food. In addition to traditional healthiness food may contain single components that may have a positive impact on our well-being. Products that are claimed to have special beneficial physiological effects in the body are usually called functional foods (Belliisle et al., 1998; Roberfroid, 1999; Hardy, 2000; Kwak and Jukes, 2001). A particular food may be made more functional by increasing or adding a potential health promoting entity. Alternatively, concentration of adverse components may be reduced or there may be a partial interchange between toxic and beneficial ingredients. Functional food may be considered as a therapeutic aid without prescription. The term nutraceutical was coined by Stephen De Felice, founder of the Foundation for Innovation in Medicine, in 1989. He defined a nutraceutical as a food, dietary supplement or medical food that has a medical or health benefit including the prevention or treatment of disease. Today the term nutraceutical is often used interchangeably with functional food. An official definition for the term functional foods is lacking in USA and Europe. According to EU concerted action “a food can be considered as functional if it has been satisfactorily demonstrated to affect beneficially one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or a reduction of risk of disease.” These functional foods may also be used as conventional foods (Diplock et al., 1999).

There are several methods of manufacturing functional foods, based either on the method used to produce them or on their purpose. Functional foods may be processed by modification or they may be fortified with different substances and the
functionality of a product can be targeted to a special disease or just to improve overall well being (Roberfroid, 1999). Information relating health effects and the ways of communicating such information are the key factors because the health effect cannot be perceived from the product itself (Urala et al., 2003). Since 1989, the nutraceutical or functional food industry has evolved into a market worth $20.2 billion in 2002. Functional beverages reached $10.35 billion sales in 2002, up 10.7% and are projected to reach $15.9 billion by 2010. A variety of functional dairy foods are conquering the market worldwide. Examples include probiotic, prebiotic, and symbiotic products, low cholesterol fresh milk omega-3-milk, low lactose, and lactose free products and milk products that can control or manage hypertension and immune functions. The market for functional foods, nutraceuticals, wellness foods, and beverages is fast growing and it is estimated that the functional food industry could almost double by 2007. An increasing sector of the public is quickly aging and purchasing functional food products. Today’s consumers are more concerned with weight loss and management, heart health, eye health, cancer, increasing energy and stamina, eye health, and improved memory. Several categories of functional foods and beverages have been developed to meet these challenges.

The scope of the present article is to provide an overview of the kinetics of lactic acid fermentation in dairy products, the technology and nutritional significance of fermented milks popular in different countries, and the therapeutic properties of probiotic organisms involved in their manufacture.

**LACTIC ACID FERMENTATION**

*Fermentation Kinetics*

Mathematical analysis of the biokinetics of functional starter cultures can yield precious information about the relationship between the food environment and bacterial functionality and may contribute to optimal strain selection and process design. This may result in better process control, enhanced food safety and quality, and reduction of economic losses. Kinetic data are needed to understand the fermentation process and to develop continuous fermentation systems. Lactic acid bacteria (LAB) have long been used in food industry as starter cultures for the manufacture of fermented meat and dairy products. The key metabolite produced during such fermentation reactions is lactic acid, which is a commercially valuable product with applications in food manufacturing and pharmaceutical industries. Both chemical and biotechnological methods are available for lactic acid production, but the lactic acid from fermentative processes has increased its market share owing to the preference of consumers to products of natural origin (Chahal, 1989). The commercial significance of dairy fermentation industry which incorporates production of cheeses, fromage frais, sour cream, etc. is well recognized and ranks second only to the alcoholic beverages (Daly et al., 1998). The processes for wood utilization can be directed toward lactic acid production (Moldes et al., 1999). The kinetics of milk fermentation by starter cultures has been studied by several research workers. Response Surface Methodology has been successfully used in modeling of acidification rate and growth of starter bacteria as a function of parameters such as fermentation temperature, fat and solid content, inoculum size, and cocci/rods ratio on the fermentation process of yoghurt (Torriani et al., 1996). The pH measurement method described by Spinnler and Corrieu (1989) is widely used in the determination of acidification kinetics during gel formation. Several research workers have tried to optimize the acidification process for manufacture of fermented products and developed models to predict the acidification kinetics. Mathematical models have been used to predict the influence of fermentation operating temperatures on cell growth rate, cell concentration, substrate utilization rate and lactic acid production rate. As for models, a number of them, purely or partially empirical, have been described in literature. Product inhibition is well-established feature of lactic fermentations.

Boonmee et al. (2003) developed a model for batch and continuous fermentation of *Lactococcus lactis* strain NZ133. The growth kinetics of *L. lactis* is predominantly influenced by lactose limitation and lactate product inhibition with the growth of this particular strain showing a relatively high sensitivity to lactate inhibition. The Leudeking-Piret equations for growth rate. As for models, a number of them, purely or partially empirical, have been described in literature. Product inhibition is well-established feature of lactic fermentations.

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$P$ is lactate concentration (g l$^{-1}$),
$P_t$ is threshold lactate concentration (g l$^{-1}$),
$P_m$ is maximum lactate concentration (g l$^{-1}$),
$q_{p,max}$ is maximum specific lactate production rate (gg$^{-1}$h$^{-1}$),
$q_{s,max}$ is maximum specific lactose utilization (gg$^{-1}$h$^{-1}$),
$s$ is lactose concentration (g l$^{-1}$),
$x$ is biomass concentration (g l$^{-1}$),
a is growth associated constant in Leudeking–Piret model (gg$^{-1}$),
b is nongrowth associated constant in Leudeking–Piret model (gg$^{-1}$h$^{-1}$),
$\mu$ is specific growth rate in Leudeking-Piret model (h$^{-1}$), and
$\mu_{max}$ is maximum specific rate (h$^{-1}$)

Common Organisms and Pathways

The group of LAB plays a central role in fermentation processes, and has a long and safe history of application and consumption in production of fermented foods and beverages. (Ray, 1992; Wood and Holzaphel, 1995; Wood, 1997; Caplice and Fitzgerald, 1999) During fermentation of lactose to lactic acid, the acidity of the milk rises and the growth conditions for microorganisms other than LAB become increasingly unfavorable (Driessen and Puhan, 1988). Reid et al. (2002) have studied the LAB in fermented foods in South East Asia. They discussed the distribution and application of LAB in various products such as fermented milk, meat, fish, vegetable, or plant products. Significant advances have been made in elucidating the genetic, biochemical and physiological basis of many of the key technological traits of these bacteria (Daly et al., 1998). LAB are constituted of heterogenous group of Gram-positive bacteria with a strictly fermentative metabolism (Temmerman et al., 2004). One of the most important functional properties common to all LAB is their ability to convert lactose and a variety of other carbohydrates into the primary end product, lactic acid, by fermentation. The ability of LAB to transform food into new products and to exert antagonistic action toward harmful microorganisms make them suitable for both domestic and industrial productions Servin (2004). The LAB involved in the production of fermented milks are distributed in several phenotypic and genotypic groups. These groups are characterized by different nutritional requirements, metabolic, cultivational, and technological properties. Several authors have reported that LAB present in fermented milks may belong to species of Lactobacillus, Streptococcus, Pediococcus, Leuconostoc, and Lactococcus genera (Aucloir and Moscouq, 1974; Cogan, 1985; Gilliland, 1985; Sandine, 1985; Schleifer et al., 1985). The role of interaction between yeasts and LAB in African fermented milks has been reviewed by Narhus and Gadaja, 2003.

There are two main fermentation pathways in LAB; the homolactate pathway in which lactic acid is the major end product and heterolactate pathway in which other compounds such as acetic acid, carbon dioxide, ethanol etc. are produced in addition to lactic acid (Huggenholtz, 1993; Cocaign-Bousquet et al., 1996; de Vos, 1996). The homofermentation and heterofermentation pathways are shown in Fig. 1. Both rods and cocci follow different pathways of carbohydrate fermentation and achieve end products, which are at least 90 or 50%, lactate in the case of homo and hetero fermentative species respectively. Lactose is transported across the cell membrane either by lactose permease system or by phosphoenol pyruvate dependent phospho transferase system (Lac PEP-PTS). Depending on the transport mechanism involved, phosphorylated lactose (Lac permease) is hydrolyzed into glucose and galactose by $\beta$-galactosidase or phosphorylated lactose is hydrolysed by phosphorylated $\beta$-galactosidase into glucose and galactose-6-phosphate. All of the dairy lactococci and some mesophilic lactobacilli operate the Lac PEP-PTS system (Fig. 2) while leuconostocs and some industrially significant thermophilic organisms; S. thermophilus and Lb delbrueckii subsp. bulgaricus possess a permease system (Fig. 3) Subsequent metabolic pathways include the glycolytic Embden-Meyerhof-Parnas(EMP), the phosphoketolase pathways for glucose, the tagatose-6-phosphate pathway for galactose-6-phosphate and the Leloir pathway for galactose (when it is not transported back into the environment). LAB containing aldolase, that is, Streptococci, Pediococci, and obligately homofermentative and facultative heterofermentative Lactobacilli have the homolactic fermentation with lactate as the exclusive end product. LAB containing phosphoketolase can be divided into two groups. The first, that is, Leuconostocs and obligately hetero fermentative lactobacilli, follows “6P—gluconate pathway” with production of equimolar amounts of CO$_2$, lactate and acetate. The others follow “bifidus pathway” with the formation of acetate and lactate in a molar ratio of 3:2.

Among the LAB, lactose metabolism in Lactococcus is probably the best understood in terms of its biochemical and genetic make-up and also in terms of its regulation. The complete metabolic pathway is known and several important genes have been characterized (de Vos and Vaughan, 1994). The genes encoding the PEP-PTS system and the tagatose pathway are all plasmid located in a single operon under the control of the repressor gene lacR. Another operon has also been identified in Lactococcus encoding a number of glycolytic enzymes The fermentative action of specific LAB strains may lead to the removal of toxic or antinutritive factors such as galactose and lactose from fermented milks to prevent lactose intolerance and accumulation of galactose (Wouters et al., 2002).

Fermentation Process and Health

Fermented milks constitute important part of our food. In ancient days, human beings realized that fermentation occurred, without, however, knowing their cause. Originally milk fermented spontaneously, and the reuse of fermentation vessels and tools contributed to a certain repeatability and stability in the fermentation process. This led to the use of specific microorganisms for the manufacture of more or less refined products. Different countries or even different parts of the same country developed their own fermented milks. The best-known product
is the thermophilic fermented milk, yoghurt, which has enjoyed increased popularity in the last three decades. The role of living microorganisms in fermented dairy products has gained considerably increased interest for both the consumer and the producer. Nature has provided a certain degree of beneficial association for humans through the activity of lactic LAB. These bacteria are widely used in foods and agricultural fermentations. In addition to their preservation, nutritional and therapeutic importance, these are known for their longevity of human life (Ram and Bhavadasan, 2002) LAB are natural inhabitants of gastrointestinal tract. These microorganisms have a number of traits which make them particularly attractive to be used as "probiotic". During fermentation of lactose to lactic acid the acidity of milk rises and the growth conditions of microorganisms other than LAB become increasingly unfavorable. Apart from the main metabolite lactic acid, fermentation microorganisms also produce bacteriostatic compounds. It has been suggested that the ingestion of LAB may counteract the effect of

Phosphoketolase pathways

HOMOFErmentation

Heterofermentation
Escherichia coli outgrowth by a number of possible mechanisms such as anti-\textit{E. coli} metabolites, detoxification of enterotoxins, prevention of toxic amine synthesis, and adhesion to the gut, thus preventing colonization by pathogenic bacteria. These aspects are comprehensively reviewed by Gurr (1983). Homofermentative LAB convert the available energy source (Lactose) almost completely to lactic acid via pyruvate to produce energy and to equilibrate the redox balance. The fermentation action of specific LAB strains may lead to removal of toxic or antinutritive factors, such as lactose and galactose, from fermented milks to prevent lactose intolerance and accumulation of galactose.

**Culture Containing Milk Products in Prevention of Gastro Intestinal Infections**

The beneficial effects of LAB and cultured milks have been attributed to their ability to suppress the growth of pathogens either directly or through the production of antibacterial substances such as lactic acid, peroxide, and bacteriocins (Kodama, 1952; Kansal, 2001). LAB are credited with imparting a number of nutritional and therapeutic attributes to fermented milk products such as improved digestion and bio availability of milk constituents, inhibition to harmful bacteria of gastrointestinal tract, suppression of cancer, alleviation of lactose intolerance, and hypocholesterolaemic effect (Mathur et al., 2000).

Supplementation of infant formulas with \textit{Bifidobacterium lactis} and \textit{S. thermophilus} has been reported to be protective against nosocomial diathroea in infants (Saavedra et al., 1994). Pediatric beverage containing \textit{B. animalis}, \textit{L. acidophilus}, and \textit{L. reuteri} has been reported to be useful in prevention of rotavirus diathroea. Treatment with Lactobacillus GG promotes systemic local immune response to rotavirus, which may be of importance for protective immunity against infections (Kalia et al., 1992). Probiotics reported to reduce antibiotic induced diarrhoea include \textit{B. longum}, \textit{B. lactis}, \textit{Lactobacillus GG}, \textit{L. acidophilus La5}, and \textit{Streptococcus faecium} and yeast \textit{Saccharomyces boulardii} (Borgia et al., 1982; Colombel et al., 1987; Surawicz et al., 1989; Nord et al., 1997). Several strains of lactobacillus have been reported to inhibit \textit{Salmonella typhimurium} (Hitchins et al., 1985). \textit{Helicobacter pylori} is recognized as the main cause of anal gastritis. Inhibition of \textit{H. pylori} NCTC 11637 by \textit{L. casei subsp. rhamnosus} and several strains of \textit{L. acidophilus} and production of antihelicobacter factors by these LAB have been reported (Kawamura et al., 1981; Lambert and Hull, 1996).
The nutritional importance and technology of traditional fermented dairy products popular in different parts of the world has been summarized in Table 1. Besides the traditional products which are popular in various countries, the development of probiotic products, dairy beverages, dietetic preparations, and dry cultured products are on progress due to the increasing demand for such value added products and convenience based ingredients. The process technology for different categories of such products is described. The major categories include cultured milks with mesophilic organisms and thermophilic milks. The organisms involved in their manufacture, metabolites including flavor compounds produced and their proposed mechanism in probiotic dairy products are given in Table 2. Dry cultured milk products, whey based cultured beverages and several dietetic preparations also find place in the booming health food market.

Cultured Milk

Cultured milk is a collective name applied to a wide range of mesophilic fermented milks with a number of characteristics in common. Mesophilic organisms are those with growth optima between 20°C and 30°C. The starters used for fermentation consist of homofermentative mesophilic LAB and of aroma producers (Driessen and Puhan, 1988). Mesophilic lactic cultures generally grow in the temperature range of 10–40°C and contain group N streptococci and/or leuconostocs (Petterson, 1988). The final taste of cultured milk is the result of a mixture of compounds present in a certain ratio. For example, the diacetyl flavor is associated with butter and buttermilk. Cultured milk should be mildly acid and slightly pricking. To meet these requirements, the content of lactic acid and carbohydrate has to be controlled during the manufacture of the product. By cooling the milk at the proper time, acidification can be limited, whereas the excess of the carbon dioxide in the product at the end of fermentation can be removed by stirring or de-aeration by vacuum.

Milk for production of cultured milk has to be heat treated in order to inactivate the native substances which are inhibitory to the LAB and to improve the structure of the final product by denaturation of whey proteins. To obtain a coagulum that can be stirred easily to a smooth and viscous product, 805 or more of the whey proteins should be denatured (Snoeren et al., 1981; Richter, 1997) this can be reached by a heat treatment of 90°C for 3 min or 85°C for 30 min (Harland et al., 1955; Hillier and Lyster, 1979).

Lowering of pasteurization temperature of milk results in decreasing firmness and retarded acidification. Ultra High Temperature (UHT) sterilized milk and other high temperature treated milks also result in lower viscosity, age-thickening and a cooked flavor in cultured milks. Homogenization of milk at 55°C and 20 MPa is sufficient to get good distribution of fat. Decreasing milk solids-not-fat content results in taste difference. The product may turn “flat” and “watery” and the defect known as “astringent” may occur. Increasing MSNF of milk leads to a “full” taste, higher viscosity, and a stable cultured milk without wheying off during cold storage.

Dry Cultured Milk Products

Extensive research has been carried out on various dry cultured products including yoghurt, acidophilus milk, kemiss, and kefir, usually combined with additives that will enhance the nutritive quality of the final product. Starters for dry cultured beverages are streptococcus thermophillus, Lactobacillus bulgaricus, Lactobacillus acidophilus (LA), “Kefir grains”, and Bifidobacterium adolescens among others (Caric, 1994). Very little scientific work has been published in this field and the developed processes are covered by patents (Pallansch, 1970; Hall and Hedrick, 1975; Shah et al., 1986; Usacheva et al., 1990). In general, the processing procedure for these products differs somewhat from that for conventional cultured products (Krsev, 1989). Milk is heated to 90°C–95°C for 20 min prior to culturing. A total solids content of 30% has been found to be most favorable. Concentration is accomplished either by vacuum evaporation or ultrafiltration. The advantage of using concentrated milk for culturing is volume reduction, and certain stimulation is provided to microorganisms in this way.
<table>
<thead>
<tr>
<th>Country/Region</th>
<th>Product</th>
<th>Technology and nutritional significance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern and eastern Africa—general</td>
<td>Fermented milks and concentrated fermented milks</td>
<td>Sourcing/coagulation of the raw milk in a smoked clay pot or bottle gourd, in warm place, whey removed for concentration</td>
<td>Bekele and Kassaye, 1987; Kerven, 1987; Shalo, 1987.</td>
</tr>
<tr>
<td>Southern and Eastern Africa—Ethiopia</td>
<td>Irgo (fermented milk). Hard fermented milk curd. Arrera (sour butter milk).</td>
<td>Milk utensils smoked by burning chips of Olea africana or Acacia busia. Similar to irgo except that there is daily removal of whey and the addition of fresh milk until the vessel is filled with hard curd. Byproduct of butter making, a beverage with milk fat content 1 to 3%, rich in protein, lactose, minerals, and vitamins.</td>
<td>Bekele and Kassaye, 1987</td>
</tr>
<tr>
<td>Middle-east Syria</td>
<td>Laban</td>
<td>Cow, sheep, or goat milk heated to 80°C, cooled to 37–40°C, innoculated with LAB or with a small amount of a previous batch of laban, for about 4 h</td>
<td><a href="http://www.fao.org/docrep/003/t0251e/T0251E04.htm#ch2">http://www.fao.org/docrep/003/t0251e/T0251E04.htm#ch2</a></td>
</tr>
<tr>
<td></td>
<td>Labaneh (laban mousafa)</td>
<td>A drained laban or drained yoghurt with combined characteristics of laban and cheese (18–23% dry matter, pH 5 to 5.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shenineh</td>
<td>Prepared by shaking laban in a special bag made of sheep skin, sour taste and a very strong aroma and is very popular in the villages.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shenglish (Sorke)</td>
<td>Made from laban (45–50% dry matter), spices and chilli added for taste.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Keshkeh</td>
<td>Made by mixing laban with fine wheat. The mixture is dried and ground into a powder.</td>
<td></td>
</tr>
<tr>
<td>West Africa—Mali.</td>
<td>Kadam (fermented milk). Sour butter milk.</td>
<td>Popular traditional beverage or thirst quenching drink. Residual milk is accumulated and allowed to sour for few hours or several days depending on season. Soured milk churned to produce butter milk.</td>
<td></td>
</tr>
<tr>
<td>Southcone countries of latin america.</td>
<td>Yoghurt.</td>
<td>Milk fermented with a mixture of <em>Lactobacillus delbrueckii</em> subsp. <em>bulgaricus</em> and <em>Streptococcus thermophilus</em>. The fermentation of lactose to lactic acid prevents milk spoilage due to the growth of contaminating bacteria</td>
<td></td>
</tr>
<tr>
<td>India and neigh boring countries</td>
<td>Dahi</td>
<td>Yoghurt-like product made from cow milk, buffalo milk, or mixture of two with addition of starter into boiled and cooked milk with mild pleasant flavor, a clean acid taste, and a yellowish creamy-white color.</td>
<td>De, 1980; Aneja, 1989</td>
</tr>
<tr>
<td></td>
<td>Mishti doi</td>
<td>Sweetened variety of dahi containing sugar, Artificial color, caramel, and jaggery.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chaas ((Buttermilk)</td>
<td>Produced by the churning of soured milk (dahi). Contains 1–2% fat and is rich in protein and lactose.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shrikhand</td>
<td>Concentrated dahi (Chakka) mixed with the required amount of sugar, condiments and flavor.</td>
<td></td>
</tr>
<tr>
<td>Himalayan region—Nepal and Bhutan</td>
<td>Lassi</td>
<td>A refreshing beverage from dahi consumed sugared or salted.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mahi</td>
<td>Traditional drink made from whole or skimmed milk dahi fermented by natural souring or by “artificial” LAB, sweetened by the addition of sugar, diluting with water. Dahi sherbet made by diluting dahi 8 to 10 times its volume with water.</td>
<td></td>
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</tbody>
</table>
drying and range from 145–175 °C (inlet air temperatures). Data have been reported for drying cultured beverages by freeze-drying on a laboratory scale. The resulting product is of good solubility and contains viable microflora after recombination. Kumar and Mishra (2003) developed mango soy fortified yoghurt powder. The nutritional and therapeutic value of yoghurt was improved by supplementing buffalo milk with soymilk, thus incorporating in the product phytochemicals. By incorporating soymilk phenyl alanine, iron, thiamin, and niacin contents of the product increased.

**Whey Based Cultured Dairy Products**

Current worldwide popularity of fruit flavored drinkable yoghurt offers an excellent opportunity for incorporation of whey into the development of cultured fruit flavored products (Singh, 2001). Proper amount of stabilizer coupled with heat processing of the ingredients and homogenization are important aspects in the manufacture of fermentable cultured dairy drinks (Singh, 2001). Kumar and Tiwari (2003) showed that good quality whey based fermented milk beverage can be made from a mixture of cheese whey and milk. The fermented beverage made by admixing 70% of cheese whey and milk. The fermented beverage contained 11.65–13.1% total solids, 11.27–11.65% total sugar, 0.66–0.78% protein, 0.2–0.3% fat, and 0.42–0.46% minerals. Mango-molke mix, a popular whey beverage marketed in Europe contains bifidobacteria culture along with whey and mango juice. Probiotic attributes of LAB may be incorporated to develop fermented whey beverages. Such beverages may be beneficial for the people suffering from gastro-intestinal tract disorders and it also provides nutritional and therapeutic soft drinks (Singh, 2001). Kumar and Tiwari (2003) showed that certain strains of *L. delbrueckii subsp. bulgaricus* could survive and compete in the human intestine. This possibility is of great importance.

### Thermophilic Fermented Milks

The term thermophilic should be reserved for microorganisms whose optimum growth temperature lies between 55 and 75°C. In dairy industry, it tends to be employed to describe cultures that are most active between 35 and 40°C. According to Bianchi-Salvadori (1981) thermophilic yoghurt starters do not form part of the natural intestinal flora of man nor do they implant themselves in the intestines. It has, however, been suggested that *L. delbrueckii subsp. bulgaricus* and *S. thermophilus* can establish themselves in the intestine and gradually dominate the natural microflora. In vitro, studies have indicated that *L. delbrueckii subsp. bulgaricus* is inhibited by bile salts (Lembke, 1963). However, these bacteria are also able to resist a high degree of acidity and so it is quite feasible that a proportion of bacteria ingested will reach the intestine in a viable state (Acott and Labuza, 1972). Mabbit (1977) supports the hypothesis that certain strains of *L. delbrueckii subsp. bulgaricus* could survive and compete in the human intestine. This possibility is of great importance.

### Table 2 Probiotic microorganism, metabolites and proposed mechanism in dairy products

<table>
<thead>
<tr>
<th>Product</th>
<th>Microorganisms involved</th>
<th>Metabolites and flavor compounds/probiotic property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidophilus milk</td>
<td><em>L. acidophilus</em></td>
<td>Maintenance of normal intestinal flora, acidophilin</td>
</tr>
<tr>
<td>Acidophilus paste</td>
<td><em>L. acidophilus</em></td>
<td>-do-</td>
</tr>
<tr>
<td>Acidophilus buttermilk</td>
<td><em>L. acidophilus</em></td>
<td>Acetaldehyde</td>
</tr>
<tr>
<td>Acidophilus yoghurt</td>
<td><em>L. acidophilus</em>, <em>S. thermophilus</em>, <em>L. delbrueckii subsp. bulgaricus</em></td>
<td>Diacetyl enhanced digestibility, prevents constipation</td>
</tr>
<tr>
<td>Biogurt</td>
<td><em>L. acidophilus</em>, <em>S. thermophilus</em></td>
<td>Removal of toxic amines, strengthening of host immune systems Reduction of serum cholesterol</td>
</tr>
<tr>
<td>Acidophilus—yeast milk</td>
<td><em>L. acidophilus</em>, lactose fermenting yeasts</td>
<td>-do-</td>
</tr>
<tr>
<td>Probiotic dahi</td>
<td><em>L. acidophilus</em>, <em>L. lactis</em>, <em>L. lactis subsp. diacetylactis</em></td>
<td>-do-</td>
</tr>
<tr>
<td>Bifidus milk</td>
<td><em>Bifidobacterium bifidum</em>, <em>B. longum</em></td>
<td>-do-</td>
</tr>
<tr>
<td>Biolacte</td>
<td><em>B. bifidum</em>, <em>L. acidophilus</em>, <em>S. thermophilus</em></td>
<td>-do-</td>
</tr>
<tr>
<td>Biokys</td>
<td><em>B. bifidum</em>, <em>L. acidophilus</em>, <em>P. acidilactici</em></td>
<td>-do-</td>
</tr>
<tr>
<td>Special Yoghurt</td>
<td><em>B. bifidum</em>, <em>L. acidophilus</em>, <em>S. thermophilus</em>, <em>L. delbrueckii subsp. bulgaricus</em></td>
<td>-do-</td>
</tr>
<tr>
<td>Cultura</td>
<td><em>B. bifidum</em>, <em>L. acidophilus</em></td>
<td>-do-</td>
</tr>
<tr>
<td>Cultura drink</td>
<td><em>B. bifidum</em>, <em>L. acidophilus</em></td>
<td>-do-</td>
</tr>
<tr>
<td>Progurt</td>
<td><em>S. lactis subsp. diacetylactis</em>, <em>S. lactis subsp. cremoris</em>, <em>L. acidophilus</em>, and/or <em>B. bifidum</em></td>
<td>Diacetyl alleviates lactose maldigestion</td>
</tr>
</tbody>
</table>

*aMetabolites; bFlavor compounds; cprobiotic property.*
importance since it would ensure the absence of putrefactive microorganisms and hence protect the intestinal health of the consumer (Dellaglio, 1984).

**Fermented Dairy Beverages**

The demand for fermented milk beverages is on the rise due to their positive health effects and nutritive value. The general requirements of fermented milk beverages are that they should be light, low calorie, for example, drinking yoghurt has 30% less energy than conventional yoghurt, refreshing (not causing thirst), tasteful of agreeable mouth feel, and smooth in texture. Milk like soft drinks mainly prepared with yoghurt and mesophilic starter cultures and nonsof drinks, for example, Ke- fir and Kumiss with alcohol production fall in the category of fermented milk beverages. Acidified milk drinks (AMDs) need a stabilizer, for example, high methoxy pectin if whey formation is to be avoided. A stabilizer can act in different ways. An increased steric repulsion between the casein micelles may be introduced, thus preventing flocculation. Alternatively, a kinetic barrier may be imposed against extensive aggregation. Casein micelles in milk (pH 6.7) are generally assumed to stay in the suspended state as the result of steric repulsive interactions between the micelles. On acidification of milk to a pH value around 4, as is done during the preparation of yoghurt, the native mechanism of stabilization of casein micelles fails. This failure is believed to be related to the collapse of the extended conformation of $\kappa$-casein chains (Holt, 1982 and De Kruif, 1998). At pH 6.7 these chains protrude from the surface of the casein micelle in order to maximise their entropy. This same tendency to maximise entropy of $\kappa$-casein chains causes the micelles to have a repulsive mutual interaction because overlap of $\kappa$-casein chains of neighboring micelles would result in a loss of entropy of the chains. This phenomenon is called steric stabilization. In order to stabilize an AMD of 8.5% milk solids (not fat), high methoxy pectin has to be added at concentrations around 0.3% w/w (Trompa et al., 2004). It was found that up to 90% of this pectin added as a stabilizer to AMDs is not directly interacting with the milk gel particles (largely consisting of casein micelles). This nonadsorbing fraction of pectin, which is, however, necessary to produce a stable AMD can be taken out of the stable system without loss of stability. Another way to decrease the amount of ineffective pectin in the AMD is to increase the energy input during the homogenization step, that is, after the addition of pectin. This will increase the adsorption rate of the pectin, and therefore allows for a smaller overall pectin concentration.

The steps in the manufacture of fermented dairy beverages include selection of a milk base with low total solids and low proteins (Rossi and Clementi, 1983; Dellaglo, 1984), selection of additives such as fruit juice concentrate, colors, artificial sweetener, sugar stabilizers, etc. taking into consideration their interactions with fermented milk components (Rasic and Kurrmann, 1988). Low temperature (e.g., <75°C) and short time treatment in order to prevent deposit formation by whey pro- teins, that is, sedimentation (Kang and Lee, 1984; Towler, 1984; Hege and Kessler, 1986). Slow acidifying starters having limited after—acidification ability; good aroma production; low ropi- ness; and low carbon dioxide production are selected for use in fermented beverages. Streptococcal cultures are the best for plain beverages. Yeast containing cultures can give the beverage excellent taste, but the gas production may lead to blowing of packages (Koroleva et al., 1983, 1984; Rasic and Kurrmann, 1988). Fermentation results in acidification down to pH 4.6 for a complete casein coagulation which prevents whey separation, too strong an acidification masks aroma, sweetness, and causes a possible destabilization of additions. Mixing of the coagulum may be carried out with a static turbine mixer or even better homogenization at lower temperature, for example, 40–45°C without pressure, Supplementary dilution may be carried out by addition of fruit juices, water, fresh milk, and vegetable juices, for example, coconut, soy, whey, etc.; carbonation of the product is also possible (Choi and Kosikowski, 1985).

**Yogurt Beverages**

Drinking yogurt is essentially stirred yogurt which has total solids content not exceeding 11% and which has undergone homogenization to further reduce the viscosity; flavoring and coloring are invariably added. Heat treatment may be applied to extend the storage life. High Temperature Short Time (HTST) pasteurization with aseptic processing will give a shelf life of several weeks at 2–4°C, which UHT processes with aseptic packaging will give a shelf life of several weeks at room temperaure.

**Cultured Buttermilk**

This product was originally the fermented byproduct of butter manufacture, but today, it is more common to produce cultured buttermilks from skim or whole milk. The culture most frequently used is *S. lactis*, perhaps also spp. *cremoris*. Milk is usually heated to 95°C and cooled to 20–25°C before the addition of the starter culture. Starter is added at 1–2% and the fermentation is allowed to proceed for 16–20 h, to an acidity of 0.9% lactic acid. This product is frequently used as an ingredient in the baking industry, in addition to being packaged for sale in the retail trade.

**Acidophilus Milk**

Acidophilus milk is traditional milk fermented with LA, which has been thought to have therapeutic benefits in the gastrointestinal tract. Skim or whole milk may be used. The milk is heated to high temperature, for example, 95°C for 1 h, to reduce the microbial load and favor the slow growing LA culture. Milk is inoculated at a level of 2–5% and incubated at 37°C until coagulated. Some acidophilus milk has acidity as high as
1% lactic acid, but for therapeutic purposes 0.6–0.7% is more common.

Another variation has been the introduction of a sweet acidophilus milk, one in which the LA culture has been added but there has been no incubation. It is thought that the culture will reach the gastrointestinal (GI) tract where its therapeutic effects will be realized, but the milk has no fermented qualities, thus delivering the benefits without the high acidity and flavor, considered undesirable by some people.

**Sour Cream**

Cultured cream usually has a fat content between 12–30%, depending on the required properties. The starter is similar to that used for cultured buttermilk. The cream after standardization is usually heated to 75–80°C and is homogenized at >13 MPa to improve the texture. Inoculation and fermentation conditions are also similar to those for cultured buttermilk, but the fermentation is stopped at an acidity of 0.6%.

**Others**

There are a great many other fermented dairy products, including Kefir, Kumiss, beverages based on *bulyaricus or bifidus* strains, labneh, and a host of others. Many of these have developed in regional areas and, depending on the starter organisms used, have various flavors, textures, and components from the fermentation process, such as gas or ethanol.

**Fermented Dietetic Preparations**

Dietetic foods are manufactured to meet particular nutritional needs of people whose normal processes of assimilation or metabolism are changed, or for whom a particular effect is obtained by a controlled intake of food or certain nutrients. They may be formulated for people suffering from physiological disorders or for healthy people with additional needs (Bender, 1975). The manufacture of dietetic-fermented milks causes problems with texture (correction with ropy starter cultures and hydrocolloids) and with flavor (correction with mild acidifying and aroma forming cultures, lactose- hydrolysis, skim-milk concentrate, fruit concentrates, sweeteners, etc.). Fermented milk products fortified with some dietary components (fiber, vitamins, phospholipids, minerals, other bioactive substances). At present, the most frequently produced fermented dietetic products are calorie reduced and dietary fiber fortified (separated and combined ones). A small number of dietetic products have been developed for adults (obesity, sports activity, etc.) and for elderly people. Only a few of the presently manufactured dietetic preparations are fermented, a factor which certainly become of more significance in the future. The use of selected intestinal bacteria starter cultures for the preparation of fermented milk products will be an expanding product area. Sarkar and Misra (2001) attempted to incorporate *Bifidobacterium bifidum NDRI* and *propionibacterium freudenreichii subsp. Shermanii MTCC 1371* along with Yoghurt- YH-3 to obtain dietetic yoghurt with enhanced nutritional and therapeutic characteristics. Dietetic yoghurt was obtained from skim milk (0.5% fat, 7.64% SNF), heated to 95°C for 30 min at 1% level each and incubated at 42°C. The dietetic yoghurt possess desirable technological and dietetic characteristics including antibacterial activity against pathogenic test organisms. The viable cell populations obtained in the product were within the range required for successful implantation in the intestine.

**FUNCTIONAL ASPECTS OF FERMENTED MILK PRODUCTS**

**Fermented Products as Vehicles for Fortification**

Fortification of foods can be an efficient tool to combat micronutrient deficiencies. The successful application of food fortification technology is based on consideration of compatibility of vehicle, fortificant, and process. Modified foods including those that have been fortified with nutrients also fall within the realm of functional foods (ADA reports). According to American Dietetic Association (ADA) functional foods including whole foods, fortified enriched or enhanced foods have potentially beneficial effect on the health when consumed as apart of varied diet on a regular basis at effective levels. Fermented dairy products, such as yoghurt and soft cheeses, have been used in iron fortification programmes (Lysionek et al., 2002). In the iron fortification of powdered nonfat dry milk, ferrous sulfate at a level of 10 ppm was found to be stable for a period of 12 months. Ferric ammonium citrate and ferric chloride at a level of 20-ppm iron in the reconstituted product gave acceptable results (Coccodrilli and Shah, 1985). Enrichment has been used interchangeably with fortification (FAO/WHO, 1994), but elsewhere it has been defined as the restoration of vitamins and minerals lost during processing (Hoffpauer and Wright, 1994).

**Probiotic Applications**

The claimed beneficial effects from the consumption of fermented milks were once a debated issue. Research conducted since the turn of the century has however, enhanced the understanding of the therapeutic effects resulting from the consumption of these products. The probiotic products are now considered helpful in maintaining good health, restoring body vigor, and in combating intestinal and other disease disorders (Mital and Garg, 1992). Most scientific papers refer to research using *L. acidophilus* and *bifidobacterium* species as dietary cultures. Nearly all probiotics currently on the market contain *Lactobacilli*, *Streptococci*, *Enterococci*, or *Bifidobacteria*. In contrast to Japan, where freeze-dried microorganisms are consumed by
a substantial part of the human population, in Europe, probiotic action toward humans is only claimed for certain fermented dairy products (e.g., yoghurts). Those species that have been extensively studied so far, with several experimental inoculation of milk for yoghurt manufacture (Prevost & Divies, 1988). Additional benefits of microencapsulation of cells include: protection of cells inside the beads from bacteriophages (Steen-son et al., 1987); increased survival during freeze drying and freezing (Kearney et al., 1990; Sheu and Marshall, 1993; Sung, 1997) The trials on man were mainly carried out with the two yoghurt bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. *L. casei*, *Bifidobacteria*, and *L. acidophilus* has also received important scientific interest, however, only a few human studies have been reported. From the technological point of view a good probiotic should be stable and viable for long periods under storage, should be able to survive the low pH levels of the stomach, be able to colonize the epithelium of the gastrointestinal tract of the host, should not be pathogenic and, last but not least, must be capable of exerting a growth promoting effect or a resistance to infectious diseases (Kalantzopoulos, 1997). Protection of probiotics by microencapsulation in hydrocolloid beads has been investigated for improving their viability in food products and the intestinal tract (Rao et al., 1989). This has been proposed for various dairy fermentations (Champagne et al., 1992a, 1992b, 1994) such as fermentation of whey (Audet et al., 1989) and continuous Kim and Yoon, 1995); and greater stability during storage (Kim et al., 1988; Reuter, 1990; Kebary et al., 1998). Many health benefits have been attributed to fermented dairy products and probiotic microorganisms (Salminen et al., 1998).

The therapeutic significance of probiotic organisms is summarized in Table 3. In order to exert positive health effects, it is generally assumed that the microorganisms need to be viable. Therefore, the International Dairy Federation (1997) has

<table>
<thead>
<tr>
<th>Clinical condition/symptoms</th>
<th>Health benefit of fermented products</th>
<th>Reference</th>
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</thead>
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<tr>
<td><strong>Lactose maldigestion</strong></td>
<td>• Viable yoghurt well tolerated by lactose-deficient subjects. L. acidophilus aids lactose digestion. • The presence of bacterial β-galactosidase in the viable yoghurt culture.</td>
<td>Morley, 1979; Gilliland, 1989; Kim and Gilliland, 1983</td>
</tr>
<tr>
<td>• Manifested by the presence of breath hydrogen derived from fermentation of the lactose in the large intestine, abdominal pain, meteorism, bloating, flatulence and diarrhea.</td>
<td></td>
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<tr>
<td><strong>Hepatic encephalopathy</strong></td>
<td>• Alteration of the intestinal microflora • A decrease in faecal urease, a lowering of blood ammonia and clinical improvement when treated individually with <em>L. acidophilus</em>, <em>Lactobacillus GG</em>, and <em>E. faecium</em>, SF68</td>
<td>Macbeth et al., 1965; Kavasnikov and Sodenko, 1967; Read et al., 1968 Loguerolo et al., 1987</td>
</tr>
<tr>
<td>• A neurological disorder where Detoxification of ammonia produced in the intestine is impaired in patients with liver failure.</td>
<td></td>
<td></td>
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<tr>
<td><strong>Side effects of radiotherapy</strong></td>
<td>• Treatment with fermented milk containing NCFB 1748 significantly decrease pelvic radiotherapy associated diarrhea • Lactobacilli and their metabolic products modify both the immune responses and antitumor activities. • Enhanced mainly by activating macrophage functions and increasing the activity of natural killer cells and T-cells, maintenance of normal intestinal flora • Help removal of dietary procarcinogens.</td>
<td>Salminen et al., 1998; Kansal, 2001</td>
</tr>
<tr>
<td><strong>Tumor or defective immune systems</strong></td>
<td>• <em>Lactobacillus</em> strains and some bifidobacteria species lower cholesterol</td>
<td>Kansal, 2001</td>
</tr>
<tr>
<td><strong>High serum cholesterol levels</strong></td>
<td>• Effect of fermented milk product on cholesterol metabolizing enzymes systems in liver • Promotion of excretion of cholesterol through faeces, • Inhibition of cholesterol adsorption by the binding of cholesterol to cells of LAB. • The promotion of excretion by binding of bile salts to lactic acid bacterial cells. • <em>Bifidobacterium</em> spp.; <em>Lactobacillus acidophilus</em> • Reduce level of toxic amines.</td>
<td>Benno et al., 1984</td>
</tr>
</tbody>
</table>

Table 3 - Fermented products and therapeutic significance
defined fermented milk as a milk product fermented by the action of specific microorganisms and resulting in reduction of pH and coagulation. These specific microorganisms shall be viable, active, and abundant (at least $10^7$ cfu/g) in the product to the date of minimum durability. Probiotics are defined as live microbial food supplements which beneficially influence the host by improving its intestinal microbial balance (Fuller, 1989; Ziemer and Gibson, 1998). Both definitions emphasize the viability of microorganisms. Whether viable microorganisms are necessary for specific health benefits remains unclear. Health benefits discussed are: alleviation of lactose maldigestion, effects on diarrhoea, immune-stimulation, antimutagenic activity, reduction of serum cholesterol, and effects on candidiasis. Many of the studies discussed here were not conducted for the purpose of determining the efficacy of nonviable bacteria or fermented milks, but killed microorganisms have often been used as placebo. The results from these studies give an indication of the activity of viable versus nonviable microorganisms and assist in defining the need for probiotics to be viable.

Fermented Milk Products With Selected Strains of Intestinal Bacteria

Fermented products containing pure strains of LA, bifidobacteria, or either of these in combination with other LAB fall in the group of probiotic foods and beverages. Important progress has been made in the study of gut bacteria in relation to their distribution, metabolic activities, and significance in health and disease. The potential beneficial roles of indigenous lactobacilli and bifidobacteria are described in many publications (Sandine et al., 1972; Poupard et al., 1973; Speck, 1976; Shahani and Chandan, 1979; Rasic and Kurmann, 1983; Goldin and Gorbach, 1984) The technology of some of these products has been given by Rasic and Kurmann (1988). A list of such products and the organisms associated with them is shown in Table 3. The concept of yoghurt as anaprobiotic carrier food has been reviewed by Lourens-Hattingh et al., 2001.

Ishibashi and Shimamura (1993) reported that the fermented milks and LAB beverages association of Japan has developed a concept of yoghurt as a probiotic carrier food has been reviewed by Lourens-Hattingh et al., 2001.

Ishibashi and Shimamura (1993) reported that the fermented milks and LAB beverages association of Japan has developed a concept of yoghurt as a probiotic carrier food has been reviewed by Lourens-Hattingh et al., 2001. The development of more probiotic products by the incorporation of probiotic organisms into the manufacturing process for well-accepted traditional dairy products is to be explored significantly. The development of convenience based dry cultured products containing viable bacteria by various techniques such as microencapsulation and the fortification of such powders or concentrated products with minerals and vitamins are gaining importance. The formulation of various health drinks and dietary preparations as value added products based on traditional fermented products will definitely bring more opportunities to the beverage industry. There is a need to intensify research to distinguish the therapeutic benefits of viable and nonviable organisms in different fermented products apart from the general nutrient enhancement achieved.

**REFERENCES**


