Recent Trends in Development of Fermented Milks

H. K. Khurana[1] and S. K. Kanawjia[2]*

[1]*Science and Technology Entrepreneurs Park, Thapar Institute of Engineering and Technology, Patiala – 147004, Punjab, India,* [2]*Dairy Technology Division, National Dairy Research Institute, Karnal - 132001, Haryana, India*

Abstract: Ever-growing consumer demand for convenience, combined with a healthy diet and preference for natural ingredients has led to a growth in functional beverage markets. Current trends and changing consumer needs indicate a great opportunity for innovations and developments in fermented milks. Scientific and clinical evidence is also mounting to corroborate the consumer perception of health from fermented milks. Probiotics, prebiotics, synbiotics and associated ingredients also add an attractive dimension to cultured dairy products. Also, 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' like fruit-yogurt beverages that are typical example of hybrid dairy products offering health, flavour and convenience. Another potential growth area for fermented milks includes added-value products such as low calorie, reduced-fat varieties and those fortified with physiologically active ingredients including fibers, phytosterols, omega-3-fatty acids, whey based ingredients, antioxidant vitamins, isoflavones that provide specific health benefits beyond basic nutrition. World over efforts have been devoted to develop fermented milks containing certain nonconventional food sources like soybeans and millets and convert them to more acceptable and palatable form thus producing low cost, nutritious fermented foods especially for developing and underdeveloped nations where malnutrition exists. Furthermore, use of biopreservatives and certain innovative technologies like membrane processing, high pressure processing and carbonation lead to milk fermentation under predictable, controllable and precise conditions to yield hygienic fermented milks of high nutritive value.

Keywords: Recent trends, fermented milks, yoghurt, probiotics, health benefits, biopreservatives.

FERMENTED MILKS -- DEFINITION AND CLASSIFICATION

Fermented foods are of great significance since they provide and preserve vast quantities of nutritious foods in a wide diversity of flavors, aromas and textures, which enrich the human diet. Over 3500 traditional, fermented foods exist worldwide [1]. Fermented foods have been with us since humans arrived on earth and of these fermented milks have long been an important component of nutrition and diet. Originally fermented milks were developed as a means of preserving nutrients [2]. The International Dairy Federation [3,4] published general standards of identity for fermented milks that could be briefly defined as follows: Fermented milks are prepared from milk and/or milk products (e.g., any one or combinations of whole, partially or fully skimmed, concentrated or powdered milk, buttermilk powder, concentrated or powdered whey, milk protein (such as whey proteins, whey protein concentrates, soluble milk proteins, edible casein and caseinates), cream, butter or milkfat—all of which have been manufactured from raw materials that have been at least pasteurized) by the action of specific microorganisms, which results in a reduction of the pH and coagulation. Recently, Brazilian law, by GMC 47/97 resolution approved by Mercosul subgroup 3, also defined fermented milk as "*products to which other food substances may be added or not, obtained by pH decrease in milk or reconstituted milk, to which other lactic products may be added or not by lactic fermentation, through the action of specific microorganisms*" [5].

Fermented milks are manufactured throughout the world and approximately 400 generic names are applied to traditional and industrialized products [6] but in actual essence the list may only include a few varieties. In the 1980s, Kurmann [7] classified fermented milks into a 'family tree' (see Fig. (**1**); Bylund [8]), which was based primarily on the optimum growth requirements of the starter cultures (i.e. mesophilic and thermophilic microflora). Taking into account the microorganisms that dominate the product, including their principle metabolites, Robinson and Tamime [9] proposed a scheme for the classification of fermented milks into

- Lactic fermentations that include (a) mesophilic type, e.g., cultured buttermilk, filmjolk, tatmjolk and langofil; (b) thermophilic type, e.g., yoghurt, Bulgarian butter-milk, zabadi, dahi and (c) therapeutic or probiotic type, e.g., acidophilus milk, Yakult, ABT, Onka, Vifit; products within this group constitute by far the largest number known worldwide;
- Yeast – lactic fermentations (kefir, koumiss, acidophilus yeast milk); and
- Mould – lactic fermentations (villi).

Certain, closely related products are manufactured from fermented milks by de-wheying; examples include labneh, skyr, ymer [9] and shrikhand [10]. Tamime and Marshall [11] have detailed the manufacturing stages of these types of fermentations. The different methods available to manufacture concentrated fermented milks are as follows: (a)

*Address correspondence to this author at the Dairy Technology Division, National Dairy Research Institute, Karnal - 132001, Haryana, India; E-mail: skkanawjia@rediffmail.com

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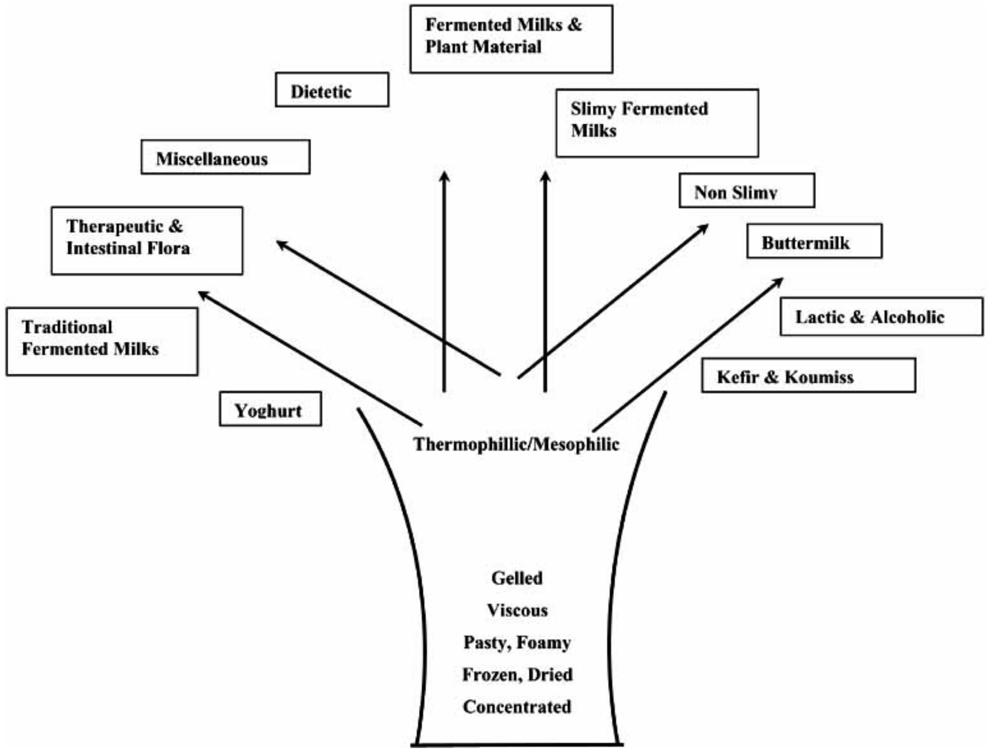

Fig. (1). The Family Tree of Fermented Milk Types [7].

cloth bag or Berge system; (b) mechanical or nozzle separators; (c) ultrafiltration (UF); and (d) product formulation [12]. Another broad classification of fermented milks given by Batish *et al.* [13] is depicted in Table **1**.

FERMENTED MILKS – GLOBAL TRENDS AND CONSUMPTION PATTERNS

According to a study by global market analyst Euromonitor international global sales of dairy products reached ¤211.5 billion [14]. The manufacture of cultured dairy products represents the second most important fermentation industry (after the production of alcoholic drinks) [1]. A dynamic category, fermented dairy drinks were reported to grow at six times the rate of total dairy growth between 1998 and 2003 in value terms. Also, probiotic drinking yoghurt was the fastest growing dairy product sector between 1998 and 2003, followed by soy milk, (spoonable) probiotic yoghurt, flavoured milk drinks with juice and fermented dairy drinks [15].

The increasing demand from consumers for dairy products with 'functional' properties is a key factor driving value sales growth in developed markets. This led to the promotion of added-value products such as probiotic and other functional yoghurts, reduced-fat and enriched milk products and fermented dairy drinks and organic cheese [14]. Another important global trend is the increasing demand for consumer convenience. Present day consumers prefer foods that promote good health and prevent disease. Furthermore, these foods must fit into current lifestyles providing convenience of use, good flavor, and an acceptable price-value ratio. Such foods constitute current and future waves in the evolution of the food development cycle [16]. There are several principal reasons for the success of fermented dairy products, which relate to nutrition and health, versatility and marketing. Scientific and clinical evidence is also mounting to corroborate the consumer perception of health from fermented milks [15].

The consumption of milk drinks and fermented products has been recently reviewed by the International Dairy Federation, shown briefly in Table **2** [17]. It is quite clear from the data that the consumption of fermented milks has generally increased around the globe over a period from 2001 to 2004. According to another report by Euromonitor [14] largest fermented dairy market till 2003 was Japan, where the leading brand Yakult is the reference product for the entire category, having been available in Japan for more than 50 years. The next most significant markets are South Korea and Brazil, followed by a number of Western European markets including US probiotic drinking yoghurts, booming on the basis of their portability, snack appeal and health claims which match those of fermented dairy drinks; offering improvement to digestive health and a boost to the immune system. In fact, Western Europe has increased in importance, becoming the second largest region for fermented dairy drinks ahead of Latin America. In 2002 Danone’s Actimel, the second largest brand globally, became the first fermented dairy drink to be launched in the US [15]. In the Indian subcontinent also, fermented milk products such as dahi (curd), *Lassi* (sweetened yoghurt drink like product)/*chhach* (buttermilk) and *shrikhand* (drained curd added with sugar and flavoring) figure prominently in people’s diet. The demand for fermented milk products is increasing and it has been estimated that about 10% of total milk produced in India is used for preparation of traditional fermented milk products. *Dahi* is an age-old indigenous

Table 1. Broad Classification of Fermented Milks. Adapted from Batish *et al*. [13]

Name	Country of Origin	Microflora
Acidophilus Milk	Australia	*L. acidophilus*
Yoghurt (Bio-ghurt)	Middle Asia, Balkans	*S. salivarius* ssp. *thermophilus, L. delbreukii* ssp *bulgaricus, Micrococcus* and other lactic acid, cocci, yeasts, molds
Kefir	Caucasus	*L. lactis* ssp *lactis, Leuconostoc* spp*. L. delbreukii* ssp *caucasiucu, Saccharomyces kefir, Torula kefir, micrococci,* spore forming bacilli
Kumiss	Asiatic steppes	*L. delbreukii* ssp *bulgaricus, L. acidophilus, Torula kumiss, Saccharomyces lactis, micrococci,* spore forming bacilli
Dahi (*dadhi*)	India, Persia	*L. lactis* ssp *lactis, S. salivarius* ssp*. thermophilus, L. delbreukii* ssp *bulgaricus, plantarum*, lactose fermenting yeasts, mixed culture (not defined)
Shrikhand (*chakka*)	India	*S. salivarius* ssp*. thermophilus, L. delbreukii* ssp *bulgaricus*
Lassi	India	*L. lactis* ssp *lactis, S. salivarius* ssp*. thermophilus, L. delbreukii* ssp *bulgaricus*
Cultured Butter Milk	Scandinavian and European countries	*L. lactis* ssp *lactis, L. lactis* ssp *diacetylactis, Lueconostocdextranicum ssp citrovorum*
Leben, Labneh	Lebanon, Arab countries	*L. lactis* ssp *lactis, S. salivarius* ssp*. thermophilus, L. delbreukii* ssp *bulgaricus, plantarum*, lactose fermenting yeasts

Table 2. Consumption of Milk Drinks and Fermented Products Including Yoghurt

Country	Kg/ head			
Year	2001	2002	2003	2004
Denmark	37.0	40.7	43.0	44.6
Germany	26.1	27.0	28.5	28.9
France	20.5	21.1	21.9	21.9
EU 15	18.2	18.1	19.3	19.6
Czech republic	14.3	14.1	14.8	14.9
Cyprus	11	10.8	10.8	10.9
Hungary	11.5	11.9	14.3	15.4
Poland	8.7	11.1	11.5	11.9
10 new EU member countries[1]	9.1	10.5	10.7	11.1
EU 25	16.7	17.5	17.9	18.2
Iceland	34.0	35.2	36.3	40.7
Norway	19.0	20.0	20.7	21.1
Switzerland	24.2	23.7	25.6	27.0
Canada	5.2	5.7	6.2	6.7
Mexico	3.1	3.2	3.8	3.9
Argentina	7.3	6.8	7.3	9.4
South Africa	3.2	3.2	1.3	1.5

[1]Including consumption of countries not mentioned. Data compiled from IDF [17].

fermented milk of India and has managed its popularity in Indian diet despite changing lifestyles and food habits. About 6.9% of total milk produced in India is utilized for making *dahi* intended for direct consumption. The volume of curd and curd products was reported to be 6.0 million tones with a market value of 120 billion rupees [10].

PRODUCT DEVELOPMENT STRATEGIES AND POTENTIAL

Understanding consumer needs and preferences are critical to successful marketing and enhancing marketing value of a product. Nutritionally improved foods with at least one nutritional improvement over the conventional counterpart have been successful in the marketplace [18]. In addition to basic technologies, modern processes lead to milk fermentation under predictable, controllable and precise conditions to yield hygienic fermented dairy products of high nutritive value [7, 12]. Cultured dairy products are an excellent medium to generate an array of products that fit into the current consumer demand for health-driven foods. Several technologies associated with culture addition, fermentation, or both are available for creating an assortment of flavors and textures in milk products [9]. It appears that accentuating the positive attributes of inherent milk constituents, incorporating health-promoting cultures, and offering a variety of flavors and textures to the consumer could enhance fermented milk consumption [14]. Product modification strategies include removal or reduction of fat, cholesterol, sodium, and calories and fortification with vitamins, calcium, fiber, active cultures and other physiologically active ingredients to align with health perceptions of consumers [16].

PROBIOTICS, PREBIOTICS AND SYNBIOTICS

Probiotics, prebiotics, and associated ingredients might add an attractive dimension to cultured dairy products for augmenting current demand for functional foods. Probiotic fermented milks, is one major segment amongst fermented milks that has tremendous potential for growth and development [14]. Milk is an excellent medium to carry or generate live and active cultured dairy products. Probiotics have been defined in many ways and the definitions have evolved especially during the last decade [19]. The definition of probiotics proposed by Guarner and Schaafsma [20] is largely adopted, which is as follows: *Oral probiotics are living microorganisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition.* This definition still very much covers what we perceive as probiotics today. Probiotics have become a major topic of lactic acid bacteria research over the past 20 years. Although, probiotics have been with us for as long as people have eaten fermented milk, but their association with health benefits dates only from the turn of the last century, when Metchnikoff drew attention to the adverse effects of some gut microflora on the host, and suggested that ingestion of fermented milk ameliorated this so-called autointoxication [21]. Probiotics and cultured dairy products have an expanding volume of scientific data and a long list of claims on their beneficial health effects. Some of these health effects, such as relief of lactose maldigestion symptoms [22-24] and shortening of rotavirus diarrhea [25-28] appear to be well documented and generally accepted. Immune modulation has also been well documented [29, 30]. For other health benefits such as treatment of irritable bowel syndrome [31], urinary tract infections [32, 33], superficial bladder cancer [34-36], nutritional management of food allergies in infants [37, 38], very promising results are available for selected probiotic strains. There are however a number of health effects that are not sufficiently substantiated to date, in particular the effects on serum cholesterol levels [39-42] and relief of constipation [43-45]. In addition to these effects, studies into the health benefits of cultured dairy products and probiotics for healthy consumers are needed as they form the major market for probiotic foods [46]. The importance of these probiotic-containing products, commonly regarded as functional foods, in the maintenance of health and well-being is becoming a key factor affecting consumer choice [47]. This has resulted in rapid growth and expansion of the market for such products, in addition to increased commercial interest in exploiting their proposed healthful attributes. Fermented milks, such as yogurt and buttermilk have received the most attention in this regard [48].

Prebiotics is another important aspect linking gut health and probiotics. This is a relatively new concept in the dairy products market. A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth, activity, or both of one or a limited number of bacterial species already resident in the colon [49]. The health effect of a prebiotic therefore resembles that of a probiotic. For a food ingredient to be classified as a prebiotic, it must:

- Neither be hydrolyzed nor absorbed in the upper part of the gastrointestinal tract;
- Be a selective substrate for one or a limited number of potentially beneficial commensal bacteria in the colon, thus stimulating the bacteria to grow, become metabolically activated, or both; and
- Be able as a consequence to alter the colonic microflora toward a healthier composition [16].

Fructooligosaccharides (FOS) are the only products presently recognized and used as food ingredients that meet these criteria. Experimental evidence suggests that certain other carbohydrate based components such as transgalactosylated dissaccharides and soybean oligosaccharides may also fit this classification [50]. At present, most searches for prebiotics are directed toward the growth of lactic acid-producing microorganisms. This is due to their purported health-promoting properties. However, it may be that future developments in the study of prebiotics may include aspects of their effect on pathogenic flora components.

Another possibility in microflora management procedures is the use of **synbiotics**, in which probiotics and prebiotics are used in combination [49]. The living microbial additions (probiotics) may be used in conjunction with specific substrates (prebiotics) for growth (e.g., an FOS in conjunction with a bifidobacterial strain or lactitol in conjunction with a lactobacillus organism). This combination could improve the survival of the probiotic organism, because its specific substrate is readily available for its fermentation, and result in advantages to the host that the live microorganism and prebiotic offer [16]. Probiotics, prebiotics, and synbiotics that may be suitable for human consumption can be incorporated into various fermented milks. A list of common probiotics, prebiotics and synbiotics is shown in Table **3** [16, 21, 49].

TECHNOLOGICAL CHALLENGES FOR FUTURE PROBIOTIC FERMENTED MILKS

The viability and stability of probiotics has been both a marketing and technological challenge for industrial producers. The technological application of probiotic organisms in fermented dairy products aims to combine the potential health benefits of the bacteria with their ability to grow in milk, resulting in a nutritionally healthy and desirable product for the consumers [49]. Unless strict demands are set on probiotic product definition and labelling their regulatory definition will remain obscure. Therefore, apart from health claims, probiotic strains intended to use in fermented dairy products should be selected on the basis of their overall effect in the products [19]. Before probiotic strains can be delivered to consumers, they must first be able to be manufactured under industrial conditions, and then survive and retain their functionality during storage as frozen or freeze-dried cultures [51], and also in the food products into which they are finally formulated [52]. The probiotic strains should also survive the gastrointestinal stress factors and maintain their functionality within the host. Additionally, they must be able to be incorporated into foods without producing off-flavors or textures; they should be viable but not growing. The packaging materials used and the conditions under which the products are stored are also important for the quality of products. Also, it is usually considered that the acceptable final population of the probiotic organisms in yoghurt at the end of the products shelf life, should be anywhere between 5 and 8 log cfu g^{-1} [53].

Table 3. Examples of Common Probiotics, Prebiotics and Synbiotics

Probiotics		
Lactobacillus spp.	*Bifidobacterium spp.*	Others
L. acidophilus	*B. bifidum*	*Streptococcus thermophilus*
L. casei	*B. longum*	*Enterococcus faecium*
L. gasseri ADH	*B. infantis*	*Pediococcus acidilactici*
L. johnsoni LA1	*B. breve*	*Saccharomyces boulardii*
L. plantarum	*B. adolescentis*	
L. casei subsp. Rhamnosus	*B. animalis*	
L. brevis	*B. thermophilum*	
L. delbrueckii subsp. Bulgaricus		
L. fermentum		
L. helveticus		
L. reuteri		
L. cellobiosus		
L. curvatus		
Prebiotics		
FOS (e.g., oligofructose and neosugar)		
Inulin		
GOS		
Lactulose		
Lactitol		
Synbiotics		
Bifidobacteria + FOS		
Lactobacilli + lactitol		
Bifidobacteria + GOS		

Compiled from Gibson and Roberfroid [49], Chandan [16] and Anuradha and Rajeshwari [21]

Many methods have been applied to enhance the survival of probiotic strains during manufacture and storage of fermented milks, and in particular yoghurt. Probiotics have a limited shelf life in conventional yoghurt. Freeze-drying is one process that not only preserves yoghurt but also helps maintain a sufficient quantity of viable probiotics [51]. During the processing and storage of freeze-dried yoghurt, oxygen content, high temperature, low pH, water activity and elevated solute concentration may all affect the viability of probiotic organisms [54]. A number of factors have been examined, such as the addition of ascorbic acid or cystein as oxygen scavengers [55, 56], supplementation of yoghurt with growth additives for probiotic bacteria such as whey powder and whey protein concentrate [56, 57], effects of acid and hydrogen peroxide [58], culture conditions and storage time and temperature [55, 59]. Immobilization of probiotic cultures in whey protein-based microcapsules can increase cell survival when subjected to extreme conditions [60]. Cryoprotectants have also been added to maintain the viability of probiotic organisms during freeze-drying. Compatible cryoprotectants may be added to media or into the yoghurt mix prior to fermentation to assist in the adaptation of probiotics to the environment. Encapsulation methods have also been applied to lactobacilli and probiotics [61] as a means of delivery of bacteria, as well as a way to increase bacterial survival, as this method creates a barrier and separates the bacterial cell from the adverse and stressful conditions of hostile environment like digestive extremes of gastric acid and bile salts. Microencapsulation of probiotics can be carried out with natural polymers to reduce cell losses during processing and storage. Microencapsulation can also be used to regulate fermentation by lactic acid producing starter cultures [62]. In recent studies it has been shown that incorporation of free and encapsulated probiotic bacteria do not substantially alter the overall sensory characteristics of yogurts and microencapsulation helps to enhance the survival of probiotic bacteria in yogurts during storage [63]. In another study in stirred yoghurt the survival of encapsulated probiotic bacteria was reported to be higher than free cells by approximately 1 log cycle [64]. Prebiotics may also aid survival of probiotic organisms during processing and storage. Shin *et al*. [65] found that the viability of commercial *Bifidobacterium spp*. in skim milk improved by 55.7% after 4 weeks of refrigerated storage when FOS were added. Recently, the combined effect of cryoprotectants, microencapsulation and prebiotics was found to increase the viability of certain selected strains of probiotic bacteria in yoghurt [66].

FORTIFICATION WITH PHYSIOLOGICALLY ACTIVE INGREDIENTS

Modern consumers are increasingly interested in their personal health, and expect the food that they eat to be healthy or even capable of preventing illness apart from being flavorful. Consumption of fermented milks has increased significantly around the world and nowadays various popular ingredients of functional significance are being incorporated into cultured dairy products to enhance their market value [16]. Since consumption of functional foods containing nutraceuticals is being highly encouraged, thus fermented milks produced with incorporation of these ingredients with specific health benefits could be of potential interest. In the presence of such new components, the gel structure and other properties of fermented milks change. Recently, a lot of research work is being carried out around the globe regarding effect of various added ingredients on fermented milks, especially yoghurt. Also, it is imperative to know a meaningful dose-benefit relationship associated with a specific fortified food. Some of these ingredients designed to enhance consumer appeal, which may be incorporated into fermented milks, include:

1) Essential Minerals and Vitamins

Certain minerals like Calcium claimed to prevent osteoporosis, cancer and control hypertension can be fortified in cultured milks. An attempt to fortify yoghurt with calcium salts revealed that yoghurt is a suitable vehicle for fortification with calcium salts and Calcium content of the fortified yoghurts could be increased with about 34.3, 37.6, and 39.4% by addition of Ca Lactate, Ca Gluconate and Ca Lactate + CaGluconate, respectively [67]. **Antioxidant vitamins** (C and E) to prevent cancer, cardiovascular disease, and cataracts as well as **multivitamin-mineral mixes** are being incorporated in fat free cultured milks to provide meal replacements for consumers within a targeted niche [16].

2) Dietary Fibers

The beneficial role of dietary fibre in human nutrition has lead to a growing demand for incorporation of novel fibres into foods [68]. There is little information about fiber fortification in cultured dairy products however various fibers like psyllium, guar gum, gum acacia, oat fiber, and soy components can be used. In one experiment, pectin and raspberry concentrate was incorporated in commercial stirred yogurt samples, increasing the consistency and it was found that yogurt with pectin was more shear stable in comparison with yogurt with raspberry concentrate [68]. In another study, seven types of insoluble dietary fibers from five different sources (soy, rice, oat, corn and sugar beet) were used to fortify sweetened plain yogurt. Fiber addition caused acceleration in the acidification rate of the experimental group yogurts, and most of the fortified yogurts also showed increases in their apparent viscosity. However, soy and sugar beet fibers caused a significant decrease in viscosity due to partial syneresis. In general, fiber addition led to lower overall flavor and texture scores as a grainy flavor and a gritty texture were intense in all fiber-fortified yogurts, except in those made with oat fiber, which gave the best results [69]. Similarly, β-glucan was used to prepare low fat yoghurt and as the amount of β-glucan increased a corresponding increase in yogurt consistency and firmness as well as a decrease in syneresis was reported. Furthermore, the micrographs revealed a denser structure with trapped water, similar to a full-fat yogurt [70]. Recently, Palacios *et al*. [71] prepared yogurt systems from whole milk, with Calcium (50 mg of calcium/100 mL of yogurt) and three levels of fiber from two wheat-bran sources. In comparison with a plain yogurt, the presence of fiber and calcium augmented the consistency, diminished the syneresis and the pH was higher.

3) ω-3 - Fatty Acids

Milk fat composition in dairy products can be altered by reducing the ratio of saturated to unsaturated fatty acids and increasing the contents of fatty acids that are more desirable for human nutrition, such as the ω-3 polyunsaturated fatty acids (PUFAs). The importance of ω- 3 - fatty acids like α-linolenic has been widely publicized because they are precursors of important long-chain fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which cannot be synthesized in the human body. Yet they are vital for the normal functioning and development of the brain, and are believed to reduce plaque formation in the arteries [72]. They are also claimed to exert cancer inhibition, anti-allergy effects and improvement in learning ability [73]. The DHA-fortified drinks are targeted at school children in Japan. Increased levels of healthy fatty acids in dairy products can be efficiently achieved by the use of selected bacteria during fermented milk manufacture [74] or the substitution of milk fat by oils with high levels of PUFAs. However, replacement of milk fat by oils with high levels of PUFAs yielded yoghurts with less firmness and higher syneresis [75]. A different possibility for increasing ω-3 PUFA content in milk is to include fish and vegetable oils or marine algae in animal diets [76, 77]. In one study, a modified milk where fat had been replaced by oils enriched in ω-3 polyunsaturated fatty acids was used for the manufacture of a set-type fermented product and no effect was found on yoghurt flavor however, product texture was adversely affected [78]. In another experiment yogurts rich in poly- and mono- unsaturated fatty acids were prepared by replacing milk fat. Fortification with oils did not have any effect on microbial growth however texture and flavor were adversely affected [79, 80].

4) Isoflavones

These are functional ingredients of a more recent interest [81], even though their commercial source, soy beans have been consumed for over 5000 yrs. Isoflavones are part of the diphenol compounds, called “phytoestrogens,” which are structurally and functionally similar to estradiol, the human estrogen, but much less potent. Because of this similarity, isoflavones were suggested to have preventive effects for many kinds of hormone-dependent diseases [82]. Isoflavones occur naturally in plants and mostly in soybeans. In nature, isoflavones usually occur as glycosides and, once deconjugated by the intestinal microflora, the isoflavones can be absorbed into the blood. At present, their possible protective action(s) against various cancers [83]**,** osteoporosis and menopausal symptoms and high levels of blood cholesterol are under investigation and, although the epidemiological evidence seems convincing, no recommended daily intake has been published [81].

5) Phytosterols and Phytostanols

Phytosterols are plant derived sterols that have similar structure as cholesterol thus these interfere with the uptake of cholesterol from the intestinal tract and are one natural way of achieving low level cholesterol in the bloodstream [84]. Jones *et al*. [85] indeed showed that the inclusion (1.7 g/day) of phytosterols into the diet of hypercholesterolemic men had the effect of lowering blood cholesterol. Plant stanols are potent hypocholesterolemic agents and a daily consumption of 2–3 g lowers low-density lipoprotein (LDL) cholesterol concentrations in hyper- and non-hypercholesteromic adults and children by 10–14% without changing high-density lipoprotein (HDL) cholesterol or triacylglycerol concentrations [86, 87]. Foods enriched with these components have therefore a great potential for cholesterol-management. Oil-based products enriched with plant stanol esters can lower low-density lipoprotein (LDL) cholesterol concentrations by 10–14% and Mensink *et al*. [88] concluded that low-fat yoghurt enriched with plant stanol esters lowers LDL cholesterol to the same extent as oil-based products within 1-week. Recently, Awaisheh *et al*. [89] prepared yoghurts from modified milk base containing three important nutraceuticals, namely omega-3-fatty acids, isoflavones and phytosterols. The cultures employed to make the yoghurts were single probiotic strains of *Lactobacillus gasseri* or *Bifidobacterium infantis* and, to achieve a short production time, a two-stage fermentation procedure was used with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* providing the rapid acidification. The nutraceuticals appeared to have no adverse effect on flavour and storage trials at 5^0C showed that the viability of the probiotic cultures was retained over 15 days.

6) Gamma-Aminobutyric Acid (GABA)

It is an amino acid that has long been reported to lower blood pressure by intravenous administration in experimental animals [90-92] and in human subjects [93]. GABA is present in many vegetables and fruits but not in dairy products. However, the effect of dietary GABA has attracted little attention as a factor that may influence blood pressure. A novel fermented milk product containing GABA was reported to lower blood pressure in people with mild hypertension [94]. Hayakawa *et al*. [95] investigated the blood-pressure-lowering effects of GABA and a GABA-enriched fermented milk product (FMG) by low-dose oral administration to spontaneously hypertensive and normotensive Wistar–Kyoto rats and it was suggested that low-dose oral GABA has a hypotensive effect in spontaneously hypertensive and finally concluded that the hypotensive effect of FMG was due to GABA.

USE OF BIOTHICKENERS -- EPS CULTURES

Exopolysaccharides (EPS) synthesized by lactic acid bacteria (LAB) play a major role in the manufacturing of fermented dairy products such as yogurt, drinking yogurt, cheese, fermented cream, milk based desserts. EPS are high molecular weight carbohydrates composed of a backbone of repeated subunits of monosaccharides [96]. D -galactose, D -glucose and L -rhamnose are almost always present, but the ratios vary considerably. Interest in EPS producing LAB has increased recently because these food grade organisms produce polymers important in determining the rheological properties of dairy products [96, 97]. EPS derived from food starters are currently being considered as an alternative in the food industry to introduce the desired texture to foods. When added to food products, polysaccharides function as thickeners, stabilizers, emulsifiers, gelling agents, and water-binding agents [98]. EPS may act both as texturizers and stabilizers, firstly increasing the viscosity of a final product, and secondly by binding hydration water and interacting with other milk constituents, such as proteins and micelles, to strengthen the rigidity of the casein network. As a consequence EPS can decrease syneresis and improve product stability [99]. Furthermore, it has been reported that some of these EPS materials contain gluco- and/or fructooligosaccharides and may generate short-chain fatty acids upon hydrolysis in the intestinal tract by the colonic microflora, and may have potential health (e.g., anti-tumor, cholesterol-lowering or immunomodulatory effects) and nutritional benefits as a prebiotic to the intestinal microflora [100, 101].

At present isolated strains of Lactococcus species and thermophilic LAB are used extensively in the manufacture of fermented milks and many EPS producing LAB have been studied extensively [96, 102-104]. They are of commercial interest as they act as biothickeners and aid in enhancing texture, mouthfeel and stability of the product. EPS producing cultures have been successfully used for the manufacture of Nordic ropy milk [105]. Scandinavian fermented milk drinks display a firm thick, slimy consistency and these rely on the souring capacity of mesophilic ropy strains of *Lactococcus lactis* subsp. *lactis* and ssp. *cremoris* and concomitant production of heterotype EPS for texture [106, 107]. Kumar [108] determined that *dahi* prepared using EPS culture had better body and texture and exhibited little syneresis. Pandya [109] reported that EPS producing cultures significantly improved the rheological properties of *dahi* and reduced syneresis.

A number of researches are being carried out to study the EPS - protein interactions**.** EPS can either be attached to the bacterial cells as capsules or found as unattached material in the growth medium. Extensive studies on microstructure and rheology of yogurt made with EPS cultures have also been carried out [108, 110]. Folkenberg *et al*. [111] produced set yoghurts with seven different exopolysaccharide-producing starter cultures and observed that yoghurts in which the EPS were associated with protein had high ropiness, low serum separation and appeared more resistant to stirring. A better understanding of the structure-function relationship of EPS in a dairy food matrix remains a challenge to further improve applications of EPS to better satisfy the consumer demand for appealing, tasty and even healthier products.

LOW CALORIE/ LOW FAT FERMENTED MILKS

With billion of dollars spent on dieting each year, consumers desire for nutritious low-cal dairy products continues to grow [112] and consumption of low - or nonfat dairy products has increased in recognition of their health benefits, and consumers' health problems [113]. Consumers of low-cal foods no longer accept compromise and with up-to-date sweetening methods, the food industry can manufacture low-cal cultured milk products, which satisfy highest organoleptic demands. Fat free and low-fat formulations for yogurt have earned highly acceptable place in consumer lifestyles that are seeking fat and cholesterol reduction. Although, the manufacture of low- or nonfat dairy products has been possible for many years, the use of fat replacers in the manufacture of dairy products is still novel. Fat replacers, which decrease the calorific value of food, can be used to solve some physical and organoleptic problems originating from low-fat levels in the final products [114].

Even though yoghurt does not have a high fat content compared with cheese and ice cream, fat replacers have been used to reduce the fat content of yoghurt [79, 80, 115-117]. Various fat replacers and replacer blends that have been used to produce low fat/ low calorie cultured dairy products and the technically developed fat substitutes are divided into 2 main types: modified starches or proteins which have good emulsifying or gel properties along with low calorie values; and modified fat/oil based products that contain bonds resistant to digestion, e.g., glycerol ethers and complex carbohydrates or fatty acid esters [12]. In one study, low calorie yoghurts were made from reconstituted SMP and seven types of starch based commercial fat substitutes (Litesse^{TM} – improved polydextrose, N – Oil^{R} II, Lycadex^{R} 100 and 200 – maltodextrin, Paselli^{R}SA2, and P-fibre 150C and 285F) added at the rate of 1.5 per cent, and these were compared with the control made with anhydrous milk fat (AMF) [79, 80]. Guven [114] concluded that yogurt samples containing 1% of inulin showed similar characteristics to the control yogurt containing 3% of milk fat. However, increased use of inulin in fat-free yogurt negatively influenced some physical properties of yogurt, i.e. whey separation, consistency and organoleptic scores.

Non-nutritive sweeteners can also be used to impart an attractive calorie reduction in fermented milks. Aspartame and Acesulfame-K individually and in combination have been used as sweeteners for the formulation of numerous low-cal and sugar free yoghurts, milk beverages, whey based beverages and cultured milk products [118-120]. Ten different plain and fruit yoghurts were prepared using sucrose, Aspartame, sorbitol, calcium saccharine, sodium saccharine, fructose, Acesulfame-K, dihydrochalcone, sucrose plus monoammonium glycyrrhizinate (MAG) and fructose plus MAG as sweeteners wherein Aspartame yoghurt was reported to be the most preferred on the basis of consumer panel [121]. Lotz *et al*. [120] determined that Acesulfame-K yoghurt permits stable sweetening during fermentation phase however Aspartame is degraded to some extent. The recommended level of each type of sweetener in strawberry yoghurt was 0.016g/100gm. Farooq and Haque [122] assessed the influence of sugar esters (.05%) of various hydrophilic-lipophilic balances on the textural properties of nonfat, low calorie yogurts over 14-d storage at 4°C. Aspartame was used (200 ppm) to sweeten a skim milk-based yogurt that was stabilized with starch (.5%). It was observed that sugar esters improved the overall quality of the yogurt. Kumar [123] advocated that 0.08% Aspartame on curd basis was most acceptable level to prepare low-cal *Lassi*. Also no effect on pH and acidity of *Lassi* was seen however there was a remarkable decrease in viscosity after Aspartame addition.

FRUITS AND FERMENTED MILKS – PRODUCT DIVERSIFICATION

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 [124]. Addition of fruit preparations, fruit flavors, and fruit purees has enhanced versatility of flavor, texture, color, variety to fermented milks and additionally fruits also have a healthy image. The association of fruits with cultured dairy products has endorsed healthy perception even more in the consumer mind. As consumers connect both these foods with health and wellness, the 2 categories of fruits and cultured milk products are typical example of hybrid dairy products offering health, flavor and convenience that will drive growth in coming years [125]. 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 [12].

Fruits are rich sources of various important phyto-nutrients namely, vitamins, minerals, antioxidants and dietary fibers. Various researchers have described the effect of fruit addition on mineral contents of yogurt [126]. 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 [127]. Processed fruits are more widely employed, they may be added to cultured milk in various forms namely fruit purees, fruit pieces, fruit syrup/juices, crushed fruit, frozen/osmodehy-drofrozen fruits, fruit preserves and other miscellaneous fruit products [12]. Shukla *et al*. [128] reviewed various methods of preparation of fruit yoghurt. Numerous other researchers have outlined processes for making fruit yoghurt with fruit concentration mainly ranging from 4-20% [129-134]. 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 [135]. 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 [131]. Ozturk and Oner [133] formulated fruit yoghurt using concentrated grape juice (CGJ) and suggested that addition of 10% CGJ provided desired sweetness and consistency to the fruit product. 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 [109]. Fruit based *shrikhand* has also been prepared wherein fruits like apple, papaya, mango were employed [136, 137]. Celik and Bakirci [138] made fruit-flavored yoghurt by adding 2.5, 5.0, 7.5 and 10.0% mulberry pekmez (MP) into milk. The addition of MP led to an increase in the fermentation time and a decrease in the viscosity of the yoghurts. 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 [139]. 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 [140]. Recently, Coïsson [141] evaluated the use of *E. oleracea* fruit juice as a natural functional colorant for yogurt. The juice of *Euterpe oleracea* Mart. fruit (Arecaceae), known as Açai 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.

WHEY BASED FERMENTED MILKS

Whey is a byproduct from cheese and casein production. 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. Through new technologies, whey and its fractions become versatile ingredients and also have high economic value. Whey products improve textural properties, extend shelf-life, emulsification and stability, improve flow properties, enhance color and taste and have been shown to provide beneficial functionality [142]. 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 B_6 and B_{12} are also present [143]. Functional properties of whey proteins, such as emulsifying, water/fat holding, foaming, thickening and gelling properties [144], also make them interesting to be used as a food ingredient. Due to their functional properties, whey solids/ whey as such could be used in conjunction with fermented milks [145]. Several studies have focused on the use of milk whey in yoghurt making and use of whey powder or whey–milk powder mixtures [146]. This process leads to the increase of milk total solid content in order to provide better consistency, texture and creaminess to the product. In other studies, replacement of skimmed milk by whey protein concentrates (WPC) and milk protein concentrates (MPC) was studied. Thus, yoghurts with different mineral and protein composition were obtained. It was observed that these components are of decisive importance in the fermentation and gelling process and also in the type of gel obtained. Yoghurts prepared with MPC and SMP, exhibit higher values of viscosity and more syneresis than yoghurts prepared with WPC. Regarding these results, WPC may be useful for drinking yoghurt production [147]. According to Penna [145] lactic beverages are a series of products including those prepared with milk and whey. Industrial lactic fermented beverages are formulated products containing yogurt, whey, fruit juice or pulp, flavor, other raw materials and allowed additives. However, yogurt microorganisms should be plenty and alive in the final product. Good quality whey based fermented milk drink containing 2.5% fat and 10% sugar was prepared by Otero *et al*. [148]. Macedo *et al*. [149] prepared low cost, probiotic whey milk beverage using buffalo milk cheese whey, cow skim milk and soymilk. Lassi like cultured milk-whey beverages have been developed using paneer whey [150] and cheese whey [151].

Other investigations examine the effects of demineralized whey powder, lactic culture concentration and mix treatment temperature on yoghurt quality characteristics. The results indicate that the addition of WPC to milk caused considerable changes in yoghurt composition, increasing acidity and influencing some taste properties. Fermentation time depended on demineralized whey concentration; it decreased in line with an increase in demineralized whey powder. Consistency increased as mix treatment temperature increased and demineralized whey powder decreased [145]. In another study whey powder was used to substitute partially the milk powder in yoghurt, which led to a slower acidification rate in yoghurts that become a little yellowish. Also, better sensory, flow properties and greater syneresis was obtained for products prepared with whey powder as compared to those prepared using skim milk powder [152]. Augustin *et al*. [153] made set and stirred yogurts using 80:20 blends of skim milk solids and sweet WPC. The resulting yogurts were reported to have higher gel strength, viscosity and lesser whey separation.

USE OF NON CONVENTIONAL FOOD SOURCES

Owing to the worldwide shortage of food attempts have been made to find alternative sources of protein, particularly for the developing and underdeveloped nations where malnutrition exists. Soybeans particularly are plentiful, relatively inexpensive and rich in protein; efforts have been devoted globally to exploit them for manufacture of more acceptable and palatable food products. Soy-based foods may provide additional benefits for the consumer for example due to their hypolipidemic, anti- cholesterolemic and antiatherogenic properties and also to their reduced allergenicity [154, 155]. Thus, incorporation of these nonconventional food sources like soybeans and different kinds of millets with fermented milks may help in increasing utilization of these non conventional food sources and producing low cost, nutritious fermented foods apart from extending the variety of fermented milks.

Several attempts have been made to incorporate soy solids to milk by various researchers world over as Soymilk based fermented milks could offer several distinct nutritional advantages to the consumer i.e., reduced level of cholesterol, saturated fat, and lactose. Soymilk is low in fat, carbohydrate, calcium, phosphorus, and riboflavin, but high in iron, thiamin, and niacin in comparison with cow's milk. Soymilk contains higher amount of protein than buffalo milk and is deficient in sulfur containing amino acids [156]. Replacing a part of milk used in making yogurts with soymilk enriches nutritional value of the product. Soymilk is characterized by beany or soy flavor which can be modified by lactic acid fermentation [157]. In one study soy yoghurt with improved sensory characteristics was prepared from a base that consisted of 22% soybean solids, 4% sucrose, 2% corn starch, 0.3% sodium citrate, water, and fermented with 5% active mixed starter culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). Comparative sensory evaluation of cow milk yoghurt and improved soy yoghurt showed non-significant difference for overall acceptability [157]. In another experiment wheat flour was totally or partially replaced with soybean for production of *Tarhana*, one of the most widely consumed fermented foods in the Middle East and Turkey. It is traditionally produced from yoghurt and wheat. Protein concentration of the product was doubled with the use of soybean, also, beany off flavor problem was diminished following a specific technique of manufacture [154]. Comparatively high sensory scores indicated that *Tarhana* was an excellent choice for soybean utilization in traditional foods. Kumar and Mishra [156] used response surface methodology (RSM) to optimize the formulation of mango soy fortified yogurt (MSFY). The independent variables were proportions of mango pulp, soymilk, and fat content of buffalo milk. The optimum formulation conditions of 7.1 kg mango pulp (18% total solids), 14.7 kg soymilk (8.2% total solids), and 78.2 kg buffalo milk (2.95% fat content and 9% solid not fat) per 100 kg were recommended for the blend formulation yielding an acceptable and good quality MSFY [156]. Recently, yogurt-like products were prepared from a combination of skim milk and soymilk (100:0, 75:25, 50:50, 25:75, and 0:100) containing saccharified-rice solution by lactic fermentation of four different cultures. The ratio of skim milk and soymilk had no significant effect on titratable acidity, also there was no significant difference in texture and overall acceptability among yogurts produced from mixed substrates and skim milk-based yogurt [155].

Mugocha *et al*. [158] prepared composites containing between 0 and 100% finger millet gruel and skimmed milk powder gruel by volume, inoculated and incubated at various temperatures. It was seen that yoghurt type bacterial cultures could be successfully used to produce a composite fermented beverage from finger millet and skim milk. Also, syneresis decreased significantly ($p < 0.05$) with increasing proportions of finger millet gruel. In another study yogurt extended with chickpea (Cicer arietinum), inoculated with *S. thermophilus* and *L. bulgaricus* was prepared and results indicated that yogurt made with 80:20 mixture and modified starch (ULTRA SPERCE M) removed syneresis and had pleasant flavor and texture characteristics. This "extended" yogurt was accepted by 80% of the judges when compared to skim milk yoghurt as control [159]. Similarly, a yogurt-like oat based functional food product “oat bio lacto” with oat beta-glucan, oat lipid unsaturated fatty acids and living probiotic lactic acid bacteria, was prepared by Beckers *et al*. [160].

APPLICATION OF INNOVATIVE TECHNOLOGIES

Membrane Processing

Membrane technologies especially Microfiltration (MF) can be profitably incorporated in the upstream and downstream processing of the fermentation broth either in discontinuous operations for separating the biomass from the produced metabolites or in continuous fermentation processes [161]. For example, MF equipment with a 0.1-μm pore size membranes are connected to the fermentor to introduce aseptically sterile thermolabile components such as vitamins or salts. Ultrafiltration (UF) has been used in the manufacture of various dairy products, including yogurts [162, 163] to increase protein in a non-fat yoghurt (NFY) mix without substantially increasing lactose content [164]. This approach eliminates defects, such as powdery taste, weak body, excessive acidity, which may occur when non-fat dry milk is used for fortification. Products of UF have high buffering capacity, which would permit reduction of harshness caused by excessive acidity. NFY is normally low in TS and may exhibit whey separation [165, 166] unless various stabilizer blends or ropy strains of yogurt bacteria [167, 168] are used. Thus UF process also reduces the use of stabilizers. A combination of ultrafiltration and diafiltration could be used to obtain enriched protein low-lactose fermented milks [169]. Kosikowski [170] added 1% spray-dried buttermilk to low lactose yogurt made from UF skim milk to increase typical yogurt flavor. In another study the quality of nonfat (9.55 to 10.39% total solids) and low fat (13.40%) yogurts fortified with UF sweet buttermilk or buttermilk powder was compared with that of a control yogurt fortified with non-fat dry milk. At a high level of fortification, ultra filtered sweet buttermilk lacked typical yogurt flavor but had good appearance and yielded the densest matrix. The addition of up to 4.8% sweet buttermilk powder to low fat yogurt mixes yielded a soft and smooth product. The NFY that were fortified with the test ingredients were comparable with the control yogurt, but yogurt fortified with Ultrafiltered butter milk was lower in carbohydrates, suggesting the potential exists to reduce caloric value [171]. Tamime *et al*. [172] proposed UF as a better alternative to the traditional *labneh* (concentrated yoghurt)-making process, which is uneconomical and unhygienic. Further, according to recent investigations made by Ozer *et al*. [173-175] on the rheology of *labneh* produced by a range of techniques for increasing TS it was concluded that UF could be used to produce gel properties similar to the traditional product and also confirmed that ultrafiltration of warm, fermented milk is a promising treatment for the manufacture of good quality *labneh*.

Application of High Hydrostatic Pressure

Application of hydrostatic pressure to fermented milks could be an alternative to the use of additives, which can adversely affect their true taste, flavor, aroma and mouthfeel. It is known that when milk is subjected to pressures '100 MPa, the casein micelle disintegrates into smaller subunits with improved aggregating properties [176]. There is also denaturation and subsequent aggregation of β-lactoglobulin in model solutions pressurized between 200 and 600 MPa [177, 178] and the viscosity of pressurized solutions of this protein is higher [179]. Nonetheless, the effect of high pressure on previously coagulated proteins is not known. High-pressure-treated milk has been successfully used to manufacture low-fat set-type yoghurt (12% total solids) with a creamy, thick consistency, requiring no addition of polysaccharides [180]. The application of high pressure to full-fat yoghurts to prevent rise of acidity after packaging has also been achieved [181]. Treatments at 200 - 300 MPa for 10 min at room temperature prevented after-acidification and help in maintaining the initial number of viable lactic acid bacteria and the yoghurt texture.

In one study, laboratory-made yoghurts were treated at high pressure (100 - 400 MPa) for 15 min at 20^0C and no significant changes in pH and total organic acids were observed after pressuring the yoghurt. Pressures over 200 MPa prevented post-acidification of the yoghurt during chilled storage. Also, pressurized yoghurts exhibited higher viscosity and amino acid contents than the untreated controls. It was thus suggested that the treatment of stirred type low-fat yoghurts at 200 MPa could be an alternative to the use of additives in yoghurt processing to prolong their shelf life, with the added advantages of improving flavor and texture of products [182]. According to Lanciotti *et al*. [183] the high-pressure homogenization treatment of milk resulted in a useful tool to obtain yoghurts having a greater variety of textures associated to a high microbiological quality. In fact, all the yoghurt types obtained by using milk treated with different levels of pressure were characterized by cell loads of the starter cultures higher than 8-log10 cfu/ml immediately after the fermentation and than 7 $\log_{10}$ cfu/ml after 60 days of storage at 4°C.

Carbonation

Carbonation is a treatment, which has sold billions of dollars worth of water and flavoring thus using this process with products of genuine nutritive value such as yogurt/cultured dairy products; new products with tremendous potential could be created. The carbonation process is cheap and safe and apparently does not have any negative effects on cultured dairy products [184]. Carbon dioxide may have both inhibitory and stimulatory effects on bacteria. These effects rely on the concentration of carbon dioxide, temperature [185], type of product [186] and type of microorganism [187, 188]. Carbon dioxide extends both the lag phase and generation time of spoilage microorganisms therefore the shelf life of the perishable foods is lengthened. Although the specific mechanism of this inhibitory effect is not exactly known, displacement of oxygen, acidification of the cell interior and interference with cellular enzymes are possible mechanisms [189]. According to Wolfe [190] the most probable major inhibitory factor of CO_2, is its ability to easily penetrate the bacterial membrane. Therefore, the intracellular pH of the bacterium decreases. As a result, the organism cannot effectively buffer this pH, and the internal enzymatic equilibria are disrupted.

Different methods can be used to add carbon dioxide to fermented milks, such as addition of carbonated water, production of a liquid drinkable yogurt by a commercial carbonation process, and the addition of metal carbonates [12]. However, these methods do not seem suitable for set/ stirred yogurt because it is a highly viscous product. Injection of gaseous carbon dioxide into the product has been suggested as a way to overcome this problem [186]. Several works have recently been carried out on the use of CO_2 for carbonation of pasteurized milk prior to the manufacture of yoghurt [191] as well as for carbonation of the cooled finished product [192, 193]. The influence of milk acidification up to pH 6.0 with CO_2 was studied on D- and L-lactic acid production, lactose consumption by yogurt starter, changes in the pH and rheological and sensory properties of yogurt [191]. A great influence of CO_2 on D-lactic acid production was not observed and yogurt manufactured from milk with lower pH values showed lower final pH values after 7 days of storage. No significant differences in sensory characteristics and viscosity between unacidified and acidified yogurts were detected. Tamime and Robinson [12] state that gas flushing (carbon dioxide or nitrogen) is a viable alternate method to extend the shelf life of fruit-flavored yogurt. Use of pressure and higher levels of CO_2 or a carbonation tank with filler device were also recommended as alternatives. This process particularly inhibits the growth of yeast and molds in yogurt. In one study, CO_2 (0.08 - 0.09 kg/cm^2) was incorporated in sweetened low fat (1.1%) plain yogurt and low fat Swiss style strawberry and lemon yogurts by way of sanitary hose. Carbonation had no significant effect on acceptability and sensory characteristics of yogurt during shelf life. However, carbonated version of each flavor was preferred by an almost equal / greater number of panelists [192]. However**,** another study reported that there was no difference between carbonated and non-carbonated samples with respect to population of yogurt bacteria. In addition, CO_2 did not ensure microbiologically safe yogurt because samples had *L. monocytogenes* and *E. coli* during storage [193]. According to Noriega *et al*. [194] carbonation of heat-treated milk prior to the *bifidobacteria* addition may contribute to reduce the contamination risk of bifidus milk by *B. cereus*. In one experiment, the suitability of milk preserved by refrigeration and CO_2 addition for the manufacture of plain yoghurt was evaluated and it was reported that the multiplication and acidification capacity of the starter as well as the evolution of organic acids were not affected by the previous refrigeration and CO_2-treatment of raw milk nor by the residual CO2 present in pasteurized milk. However, refrigeration enhanced the production of ethanol and diacetyl. Also no differences on sensory properties were detected thus it was concluded that refrigerated milk acidified with CO_2 could be satisfactorily used in the manufacture of yoghurt [195].

Biopreservation

Cultured dairy products are generally perishable commodities. Over the years, cultured dairy products have developed different identities and appeals. The recent increase in their popularity has been attributed to the introduction of fruits, stabilizers, flavors, coloring agents and unique starter culture combinations into the manufacture of the product [196]. However, these additives and techniques have also increased processing control demands, thus requiring more stringent manufacturing practices. The low pH of yogurt offers a selective environment for the growth of acid tolerant yeasts and molds [197].

Benzoates and sorbates are permitted additives to inhibit yeasts and molds and therefore may be found in yogurt [198, 199]. These acid tolerant organisms, however develop resistance to chemical preservatives and also, in recent years consumers have been demanding a reduction in the use of chemicals in their foods and beverages because of potential health risks [200-202]. Use of biopreservatives is one important alternative technology that could be used to extend the shelf life of ready to consume fermented milks and preserve the freshness, flavor, texture and nutrient value of these products [203]. The antimicrobial system possessed by lactic acid bacteria offers scope for the development of an effective natural preservation process. The low molecular weight compounds elaborated by LAB, capable of exhibiting antagonism are termed as bacteriocins [204]. These have inhibitory effect over spoilage organisms in yoghurt, cheese and other fermented foods. These lactic acid bacteria not only inhibit growth of spoilage organisms, as a result increase shelf life of the product, but also add therapeutic value to fermented foods. Various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocin or bactericidal proteins are produced during lactic fermentations [205-211]. The bacteriocins produced by LAB are considered as safe GRAS and have arisen a great deal of attention as a novel approach to control pathogens in foodstuffs [212]. The potential application of bacteriocins as consumer friendly biopreservatives either in the form of protective cultures or as additives is significant. Besides being less potentially toxic or carcinogenic than current antimicrobial agents, lactic acid bacteria and their by products have been shown to be more effective and flexible in several applications [213].

Research on bacteriocins from lactic acid bacteria has expanded during the last decades, to include the use of bacteriocins or the producer organisms as natural food preservatives. Lactic acid bacteria have an essential role in most food and beverage fermentation processes and selective isolated strains can positively have impact on their use as starter cultures for fermented milks, with a view to improving the hygiene and safety of fermented milk so produced [212, 214]. Nisin an important bacteriocin, is a polypeptide antibacterial substance or bacteriocin produced by the fermentation of a modified milk medium with *Lactococcus lactis ssp. lactis*. Nisin has an inhibitory effect against a wide variety of gram-positive food-borne pathogens and spoilage microorganisms [215] and can also act on several gram-negative bacteria when the integrity of their outer membranes is disrupted [216, 217]. Nisaplin, the commercial product containing 2.5% pure nisin A, is being legally used in more than 50 countries for specific food applications [218]. Nisin at a concentration of 100 IU/g extended the shelf life of yoghurt from 3-7 days without significant change in flavor, body, texture and consistency [219]. Rajmohan and Prasad [220] found nisin or nisin producing organisms capable of producing 1000 IU/ g to be effective in controlling the lipolysis during storage in dahi at 25^{0}C. Incorporation of 15 RU/g nisin into dahi retained all its desirable characteristics upto 35 days at 15^{0}C [221]. Recently, Savadogo *et al*. [212] isolated eight strains of lactic acid bacteria producing bacteriocin from Burkina Faso (in Pakistan) fermented milk samples. These strains were identified to species: *Lactobacillus fermentum*, *Pediococcus spp*., *Leuconostoc mesenteroides subsp. mesenteroides*, *Lactococcus.* Isolated bacteriocin exhibited antibacterial activity against *Enterococcus faecalis* 103907 CIP, *Bacillus cereus* 13569 LMG, *Staphylococcus aureus* ATCC 25293, *Escherichia coli* 105182 CIP using the agar drop diffusion test.

Propionic acid bacteria (PAB) also produce metabolites with antimicrobial activity such as propionic acid, acetic acid and diacetyl [222]. Propionic acid bacteria have also been found to produce antiviral peptides and several bacteriocins [223, 224]. The biopreservative Microgard is skim milk pasteurized after fermentation with *P. freudenreichii* ssp. s*hermanii* [196]. It has been approved by the FDA for use in products like cottage cheese and yogurt. Microgard inhibits some fungi and Gram(–) bacteria, but not Gram(+) bacteria [225]. According to Daeschel [222] about 30% of the cottage cheese produced in the United States was made with Microgard as a preservative. Microgard inhibited Yeast and mold growth in yogurt even at 0.5% level and the MicroGARD™ supplemented yoghurt were also protected from spoilage by Gram negative psychrotrophs which grow out following pH increase as a result of yeast growth [226]. Another commercial product is BioProfit, which contains *Lactobacillus rhamnosus* LC 705 and *Propionibacterium freudenreichii* JS. Used as a protective culture (10^7 cells per gram) the product is reported to inhibit yeasts in dairy products and *Bacillus* spp. in sourdough bread [227].

From the various screenings that have been carried out it is clear that LAB and PAB can produce a number of antimicrobial compounds, and most of them still await complete identification [228]. Thus, besides the challenge in isolating and characterizing the inhibitory compounds, there are also opportunities in exploiting them in practical applications such as food and feed preservation and medicine. This will require detailed insight into the molecular biology of these organisms and the mechanisms that regulate the production of bacteriocins and other antimicrobial compounds.

CONCLUSION AND FUTURE PERSPECTIVE

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

Over the past century, voluminous scientific knowledge has been well established regarding the technological aspects of fermented milks, including the physiology of starter cultures and related probiotic microflora. However, over the coming years the possible research areas may include the following aspects:

- Special emphasis on research in arena of starters and their functionality is required; specially in view of natural biodiversity that still exists in food grade microorganisms as starter cultures are the heart of fermented milk industry. It is also very important to preserve this pool for future application. Thus, it is necessary to have better understanding of enzymic pathways in these starters in order to be able to select strains with specific, desired characteristics.
- Appropriate international definition(s) of yoghurt and other fermented milks including other probiotic products are required.
- More emphasis is required to get a clear understanding of relationship between food, intestinal bacteria, human health and disease in the field of probiotics along with properly designed clinical studies to establish the proper health benefits to humans. Many a times *in vitro* results cannot be found *in vivo*, and observations reported in animals cannot be translated directly to humans; there are problems in generalizing the results given types of microorganisms used thus more number of clinical studies should be conducted on humans of different races in different countries to properly substantiate the health benefits to humans in general.
- Newer molecular research tools, better process formulation technologies for enhanced probiotic stability and functionality along with biosafety evaluation of probiotics used for human consumption are other major thrust areas. New product categories, and thus novel and more difficult raw materials with regard to technology of probiotics, will certainly be the key research and development area for future functional food markets.

There are now products with complete supplementation offered as medical foods, as well as healthy products for people who have problems obtaining all the nutrients they need. It is clear from the literature that new kinds of fermented milks containing various nutrients are being tested as curatives for specific diseases and are approaching medical food effectiveness in conventional food format and will continue to be introduced to the food supply. The occurrence of diet-related diseases of deficiency and excess, points to the importance of the development of functional foods (science). Functional food science must be viewed world over beyond the short-term commercial prospects and should be considered for long-term research and development.

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