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Fortification of Yoghurt with Health-Promoting Additives: A Review

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ABSTRACT

Health benefits associated with fermented milk products can be attributed to the bacterial starter culture or to the dietary adjuncts added after to the fermented product. Infectious diseases are the biggest problems for human beings in today's world. Every year gastrointestinal infections lead to significant morbidity and mortality worldwide. Consumption of fermented dairy products improve host metabolism by maintaining balance of the intestinal micro flora and may thus have a positive effect on the host. Improved digestion of lactose, aid in control of serum cholesterol levels, antagonistic action toward pathogens and control of certain types of intestinal cancer are the primary potential health benefits associated with fermented products. Among all milk fermented products, yoghurt is a well-known and more common product and has more acceptability as compared to other products in the world. Yoghurt is easily digested (because milk protein, fat and lactose components undergo partial hydrolysis during fermentation), has high nutritional value and is a rich source of carbohydrates, protein, fat, vitamins, calcium, and phosphorus. This part of the review presents some of the cases of fortification of yoghurt along with their benefits and drawbacks. The practice of fortification of yoghurt with health promoting additives is gaining popularity and is expected to fulfill more nutritional needs also.

INTRODUCTION

Yoghurt is a product of the lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. In some countries less traditional microorganisms, such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii* ssp. *lactis*, are sometimes mixed with the starter culture [1-4]. Other lactic acid bacteria, in particular those from the genera *Lactobacillus*, *Streptococcus*, *Leuconostoc*, and *Bifidobacterium* [5] can be combined with yoghurt starters to produce fermented milks with specific desirable characteristics, such as new flavor [6]. In addition, some lactic acid bacteria are included for their probiotic properties. "Probiotic bacteria are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host beyond inherent basic nutrition" [7]. Although fermented milk products such as yoghurts were originally developed simply as a means of preserving the nutrients in milk, it was soon discovered that by fermenting with different microorganisms, an opportunity existed to develop a wide range of products with different flavors, textures, consistencies and more recently, health attributes. Like milk, yoghurt is a healthy and delicious food due to its high nutritive and therapeutic value [8]. Yoghurt is an excellent source of calcium and high-quality protein. But it contains very little iron, which is common among all dairy products [9]. Yoghurt is considered as healthy food due to its high digestibility and bioavailability of nutrients and also can be recommended to the people with lactose intolerance, gastrointestinal disorders such as inflammatory bowel disease and irritable bowel disease, and aids in immune function and weight control [4,10]. Yoghurt is valued for controlling

the growth of bacteria and in curing of intestinal disease like constipation, diarrhoea and dysentery, anti-carcinogenic effect and lowering of blood cholesterol [11]. Because of these health benefits associated with yoghurt consumption, there is an increasing trend for yoghurt consumption and is the fastest growing dairy sector in the market, in particular, standard yoghurt and yogurt drinks [12]. The market now offers a vast array of yoghurts to suit all palates and meal occasions. Yoghurts come in a variety of textures (e.g. liquid, set and stirred curd), fat contents (e.g. regular fat, low-fat and fat-free) and flavors (e.g. natural, fruit, cereal, chocolate), and can be consumed as a snack or part of a meal, as a sweet or savory food. This versatility, together with their acceptance as a healthy and nutritious food, has led to their widespread popularity across all population subgroups [4]. Moreover, yoghurt represented \$51 billion in global spending in 2011, whereas Switzerland and Saudi Arabia were among the leading per head yoghurt consumers globally that accounts for 28.8 and 22.1 kg of yoghurt per head per year in 2008 [13]. The practice of fortification of yoghurt with iron is gaining popularity and is expected to help fulfill nutritional needs. Yoghurt is generally considered as a safer product and its unique flavor is also so much appealing that consideration is being given by nutritionists to incorporate inexpensive source of nutrients to make it an almost complete food [14].

GENERAL MANUFACTURING PROCEDURE OF YOGHURT

Manufacturing of yoghurt is an ancient technique, which dates back to thousands of years, and the knowledge has transferred from generation to generation. However, during the last few decades, it has become more rational due to improvement of various fields such as microbiology, biochemistry and food engineering. Today it is a complex activity combined with art and science [15]. The generalized process of yoghurt making is comprised of modifying the original composition of milk, pasteurizing the yoghurt mix, fermentation at thermophilic temperatures (40-45 °C), cooling and addition of fruits and flavors. The production steps in manufacture of stirred- and set yogurt are illustrated in the **Figure 1**.

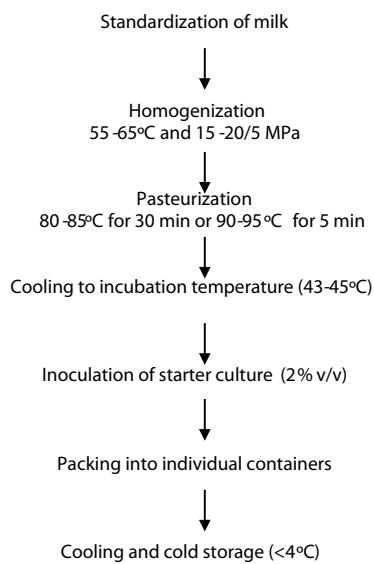


Figure 1. Manufacturing process of yoghurt [16].

NUTRITIONAL PROFILE OF YOGHURT

Yoghurt is a highly nutritious and easily digestible dairy product which is a rich source of more than ten essential nutrients in particular, certain minerals and vitamins. The nutritional composition of yoghurt can be varied according to the strains of starter culture used in the fermentation, type of milk used (whole, semi or skimmed milk), species that milk is obtained (bovine, goat, sheep), type of milk solids, solid non-fat, sweeteners and fruits added before fermentation as well as the length of the fermentation process (**Table 1**).

HEALTH BENEFITS OF YOGHURT

Health benefits of yoghurt include providing immune support for fighting against infections or cancer, providing a healthy replacement with good bacteria in the intestinal tract following antibiotic therapy, reducing occurrence of diarrhoea in humans, aiding in lowering cholesterol and improving the symptoms of lactose intolerance [18,19]. Numerous health benefits have been associated with the use of yoghurt including:

Lactose Intolerance

Lactose is the main carbohydrate found in milk and dairy products and is digested by the enzyme lactase into its absorbable components - glucose and galactose - which are then used as fuel by the body. Lactose mal-digestion or lactose intolerance occurs when insufficient amounts of the enzyme lactase are available in the gut to digest lactose [20]. This results in undigested lactose passing into the large intestine where it can be fermented by the bacteria in the colon causing adverse gastrointestinal symptoms

in some individuals, such as flatulence, diarrhoea and abdominal pain [21,22]. This problem is known as lactose intolerance and is defined as “the occurrence of symptoms after persons with clinically diagnosed lactose malabsorption (lactose intolerance) consume a trigger amount of lactose”. Although the lactose content of yoghurt is naturally lower than milk, research has shown that the lactose that is present in yoghurt is better tolerated by individuals with low production of lactase (hypolactasia) than lactose in an equivalent quantity in milk. This may be due to several factors including the activity of live bacteria in the yoghurt or the digestive action of other enzymes on lactose such as bacterial β-galactosidase. The increased viscosity of yoghurt compared with milk has also been suggested as a potential explanation, as this may result in slower emptying from the stomach and thus a longer transit through the gastrointestinal tract, which in turn may improve the absorption and reduce the amount of lactose present in the colon [23]. Yoghurt is well tolerated by individuals who have lactose intolerance and is important for those suffering from lactose intolerance who often excludes all dairy foods from the diet which can lead to poor calcium intake. Interestingly, studies have suggested [20] that even in subjects with lactose intolerance, consumption of small amounts of any dairy food, if combined with a meal, is often well tolerated and regular consumption may even reduce symptoms.

Table 1. Nutritional composition of different varieties of yoghurt (per 100 g).

Component	Whole milk yoghurt	Low fat yoghurt	Non fat yoghurt	Greek-style yoghurt	Drinking yoghurt
Energy (kcal)	79	56	54	133	62
Protein (g)	5.7	4.8	5.4	5.7	3.1
Carbohydrate (g)	7.8	7.4	8.2	4.8	13.1
Fat (g)	3.0	1.0	0.2	10.2	Trace
Thiamin (mg)	0.06	0.12	0.04	0.12	0.03
Riboflavin (mg)	0.27	0.22	0.29	0.13	0.16
Niacin (mg)	0.2	0.1	0.1	0.1	0.1
Vitamin B6 (mg)	0.10	0.01	0.07	0.01	0.05
Vitamin B12 (mg)	0.2	0.3	0.2	0.2	0.2
Folate (μg)	18	18	8	6	12
Carotene (μg)	21	Trace	Trace	Trace	Trace
Vitamin D	0	0.1	Trace	0.1	Trace
Potassium (mg)	280	228	247	184	130
Calcium (mg)	200	162	160	126	100
Phosphorus (mg)	170	143	151	138	81

Source: The Dairy Council, 2013 [17].

Weight Control

Obesity is an increasing public health problem in the UK, with one in five adults now classed as obese. Fears about weight gain are often cited as a reason to cut down on the consumption of milk and dairy products. However, contrary to popular belief, research has shown [24] that people who consume milk and dairy foods are likely to be slimmer than those who do not. Studies have also shown [24] that consumption of milk and dairy foods as part of a calorie controlled diet is associated with increased weight loss, particularly from the abdomen, since excess fat around the trunk region of the body is associated with greater risks to health. The precise mechanisms involved are unclear but studies [25] suggest they are likely to involve calcium found in milk, yogurt and other dairy foods.

Digestive Health and Immunity

Healthy levels of good bacteria promote digestive health and boost the immune system by creating an environment where bad bacteria are unable to grow [26].

Better Absorption of Nutrients

The strains of good bacteria found in freshly made yoghurt can also help the body digest and absorb important nutrients found in your food. This makes yoghurt consumption more beneficial than drinking milk. Once probiotic bacteria are eaten, the bacteria helps with pre-digestion and causes nutrients in the food to be in a better state for digestion and absorption into the body.

Probiotics

Probiotic organisms are ‘live micro-organisms which, when consumed in adequate amounts, confer a health benefit on the host’ [27]. To observe a positive health effect of their consumption, a minimum level of live microorganisms is required; this level, depending on the strains used and the required health effect, is usually between 10^8 and 10^{11} cfu/d [28]. Fermented dairy products such as yogurt have the ability to support the viability of probiotic cultures and therefore provide a suitable vehicle for their delivery into the body, and are easily incorporated into the diet. Increasingly, yoghurts have been prepared with probiotic microorganisms such as *L. acidophilus* with varying viability over a range of shelf life [10]. Non-pathogenic (non-harmful) intestinal bacteria play an important role in the normal functioning of the gut and improve resistance to disease; however factors such as changes in lifestyle, diet, increased stress and consumption of antibiotic medications can all lead to changes in the balance of gut bacteria [29].

Reduced numbers of beneficial bacteria such as *Lactobacilli* and *Bifidobacteria* and increased numbers of pathogenic bacteria may cause health complaints and infections and may pre-dispose individuals to a number of life threatening diseases such as bowel cancer and inflammatory bowel disease^[30]. In these situations, probiotics can exert a positive effect on health by redressing the balance of health-promoting and pathogenic bacteria in the gastrointestinal tract^[31,32]. The micro-organisms most commonly used as probiotics are lactic acid bacteria such as *Lactobacilli* and *Bifidobacteria* which are both found naturally in the gut. Numerous health benefits have been connected to the use of probiotics including following:

Diarrhoea

Diarrhoea often results from disturbances to the natural balance of gut microflora through infection with pathogenic strains of bacteria. The treatment of diarrhoea with probiotics has traditionally been one of the main application areas for probiotics. The best documented evidence exists for shortening the duration of a form of diarrhoea in children known as "rotavirus diarrhoea" by selected probiotic strains^[33]. Different strains, including *L. reuteri* SD2112, *L. rhamnosus* GG, *L. casei* DN-114 001, and *S. cerevisiae* (boulardii) Lyo, tested in controlled clinical trials decreased the severity and duration of diarrhoea. Several meta-analysis of controlled clinical trials have been published that concluded these probiotics are safe and effective^[32]. Prevention and treatment of diarrhoea after the use of antibiotics, particularly in the elderly, has also been connected with certain probiotic strains e.g. *L. acidophilus*, *L. bulgaricus* and *B. longum*^[34]. Unfortunately many of the trials in this area have been poorly controlled and used low numbers of subjects. Therefore further studies with improved design are needed to determine the full effect of probiotics on the treatment of diarrhoea.

Immunologic Effects

The immune system is responsible for protecting us from infection and disease. Some sub groups of the population may have weak immune function such as infants, the elderly, surgical patients, trauma victims, people under stress and HIV positive individuals which reduces their natural protection from illness^[35].

Probiotics have been connected to improved immune function in these groups^[36], and the results of human studies indicate that yogurt containing probiotic strains of bacteria can enhance immunity and reduce the incidence of conditions such as cancer, gastrointestinal disorders and symptoms of allergy, although further research is required.

Inflammatory Bowel Disease

The term Inflammatory Bowel Disease (IBD) includes disorders such as Crohn's disease, ulcerative colitis and pouchitis^[37]. These conditions are usually chronic (long term) and go through periods of exacerbation followed by periods of remission throughout the life of sufferers. The cause of the disease is unclear, but several theories suggest it may be due to abnormal responses to certain bacteria in the gut. The current treatment for IBD involves suppression or modulation of the immune system with steroid medications in order to induce remission, or surgery to remove the diseased section of the bowel. However both treatments result in numerous complications for the patient. At present treatments do not address the contribution of the intestinal bacteria (microflora)^[38] to the condition, which is an area where probiotic intervention may prove beneficial. In theory, probiotics have the potential to reduce the immuno-inflammatory responses induced by bacteria that may be responsible for damage to the intestines seen in these conditions.

Treatment with the probiotic *Escherichia coli* Nissle 1917 was reported to be as effective as the standard drug treatments for maintaining remission in ulcerative colitis^[39]. Additional studies have found benefits with supplementation of mixtures of several probiotics in inducing remission and preventing relapse of pouchitis, ulcerative colitis and Crohn's disease.

Some practitioners now believe that the evidence is strong enough to prescribe certain probiotics for treatment of inflammatory bowel disease as an alternative to current drug treatments. However most feel that further investigations are required to determine the full therapeutic potential of probiotics before changes are made to current treatment procedures.

Irritable Bowel Syndrome

Irritable bowel syndrome (IBS) is a gastrointestinal condition characterized by abdominal pain, bloating, excessive gas, constipation and diarrhea^[40,41]. The type and severity of symptoms experienced varies widely between individuals and, in general, the condition is poorly understood^[42]. No test currently exists for IBS and therefore it can only be diagnosed by excluding the presence of other gastrointestinal abnormalities^[43]. IBS sufferers are commonly found to have abnormal gut bacteria and this is thought to contribute to the development of the disease^[44].

For this reason, the use of probiotics to redress these abnormalities has been suggested as a potential treatment. To date, there have been few studies involving probiotics and IBS, possibly because IBS sufferers are a very diverse group and are therefore difficult to study. The studies that have been conducted so far have produced conflicting results, some with very real improvements in symptoms and some with no change^[45]. Although the use of probiotics in the treatment of IBS is currently not justified; more research in this area is certainly warranted because abnormalities seen in the gut bacteria (microflora) of IBS sufferers suggest that a probiotic approach will ultimately be justified. Health benefits associated with the consumption of probiotics are given in **Figure 2**.

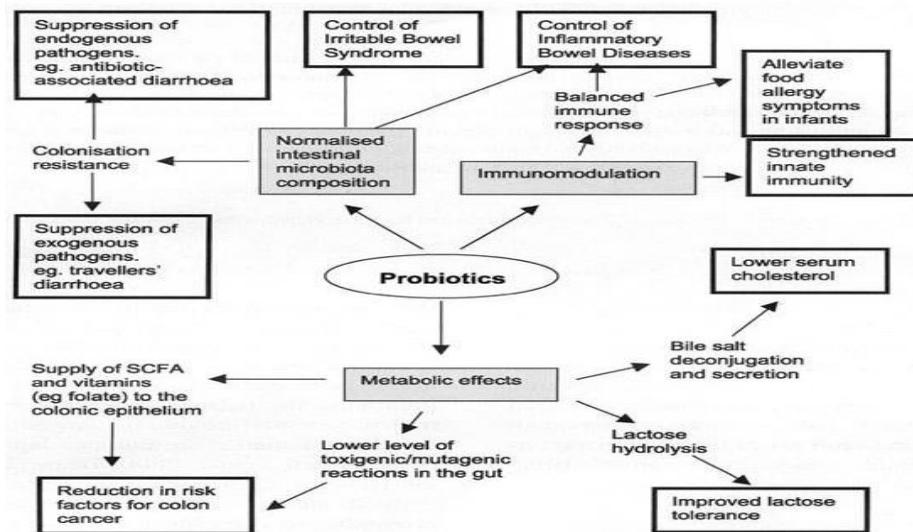


Figure 2. Proposed health benefits associated with the consumption of probiotics [47].

Sensory Properties of Probiotic Yoghurt

Yoghurt is the most used medium to incorporate probiotic bacteria in foods and, in this regard, research studies [46] have optimized its sensory qualities to render a palatable product to consumers. Hekmat and Reid [47] conducted consumer taste panel evaluations to compare the sensory properties of probiotic and standard yogurt. The results showed that the appearance, flavor, texture, and overall quality of probiotic 1% fat yogurt were comparable and similar to standard 1% fat yogurt. Thus, *L. rhamnosus* yoghurt supplemented with *L. reuteri* has sensory attributes suitable for human consumption. The yoghurt containing *L. paracasei* exhibited a rich, smooth, and traditional taste, not too acidic, similar to its regular form, showing good sensory acceptance among consumers.

Fortification of Yoghurt with Health Promoting Additives

In a recent study, yoghurts were enriched with fiber obtained from the non-edible part of asparagus shoots and fiber obtained from all methods of its processing was found equally prospective for yogurt enrichment [48]. By-products, rich in fiber and bioactive compounds, contribute about 50% of the total weight in the asparagus processing industry, which emphasizes their potential contribution to human nutrition. Hekmat and McMahon [49], found that the oxidized flavor scores of iron-fortified yogurts were slightly higher than control yogurt, and there was no enhancement in metallic, bitter, or other off-flavors. There was no detectable significant difference in the appearance, mouth feel, flavor, or overall quality of fortified and unfortified yogurts, as observed by the consumer panel. It implied that all yoghurt samples were appreciated by the consumer panelists signifying yoghurt as an appropriate medium for iron fortification. Plain whole yoghurt contains about 120 mg calcium per 100 g [50]. Fortification of yoghurt with calcium is expected to be able to address individuals' requirements that are at risk of calcium deficiency related disorders. Even when individuals do not consume large amounts of dairy products to meet their calcium requirements, fortification will supply required amount of calcium in 1 or 2 servings. Fortification of low-fat yoghurt with calcium gluconate [51] is possible with minor physical and chemical changes and no effect on organoleptic properties of the yoghurt [50]. The increasing emphasis on use of natural food additives in diet has promoted the use of honey, which has been gaining interest as a substitute sweetener because of its wholesome image [52,53]. Low pH (approximately 3.9) and ability to decrease sourness of solutions make it an attractive additive for acidic products such as yoghurt. However, combinations of honey with yoghurt are comparatively rare [54] because of its inhibitory effects on lactic starter cultures [54,55]. Factors related to antibacterial nature of honey are not completely understood [56], and are expected to be because of "high sugar content" (limiting water available for proper growth of microorganisms), the "relatively high acidity, the presence of organic acids, and the presence, at low concentrations, of hydrogen peroxide" [57]. Floral source of the honey is an important factor influencing its antimicrobial characteristics [58]. Studies have shown that during storage at 4 °C, the characteristic microorganisms (that is, *S. thermophilus* and *L. bulgaricus*) in yoghurt are not significantly affected by the presence of honey at 1.0% to 5.0% (w/v) [59]. pH and lactic acid levels of the final products were found unaffected by honey, and it highly improved the sensory characteristics of the final product, with approximately 3.0% (w/v) of honey, without having a negative effect on the lactic acid bacteria.

Fortification of non-fat yoghurt with whey protein isolate (WPI) has also been tried [60] and fortification of yoghurt with sodium caseinate and yogurt texture improver (TI) has also been done. It was observed that yoghurt with WPI did not have desirable sensory properties and the descriptive panel indicated that yoghurt with WPI had the lowest fermented flavor attribute. For the last several decades, marine lipids have received growing interest because of their valuable health effects [61]. Many studies have demonstrated that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have a positive effect on myocardial infarction and on immune and eye functions [62-64] and in neurological diseases, for instance depression and Alzheimer's disease [65]. The healthy image of EPA and DHA and the wide consumption of dairy products such as yoghurt support the possibility of use of

yoghurt as a vehicle for increasing consumption of fish oil. However, due to the presence of high content of polyunsaturated fatty acids, foods with fish oil are prone to oxidation that might lead to the development of undesirable fishy and rancid off-flavors and may even promote cardiovascular diseases [66-68]. In a separate study [2], an algal oil emulsion was added to yoghurt mix flavored with a strawberry fruit base, to supply 500 mg ω-3 fatty acids per 272g serving of yoghurt. Cashew apples, a rich source of vitamin C [69] was used as a nutritional additive in yoghurt making [70]. The yoghurt into which cashew apple had been added “compared favorably” with the reference sample (commercially available Fan Milk yoghurt) in terms of all the attributes evaluated and there was no significant difference. Hassan [71] manufactured a yoghurt using concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5% of aqueous garlic extract. According to their results, after 15 days of storage, the resultant yoghurt in all different concentrations of garlic had higher values of sensory, texture and aroma than control. Foda [72] prepared set yoghurt using buffalo milk and supplemented with turmeric powder at different concentrations of 0.1, 0.25, 0.50, 0.75 and 1%. They found an increase in the firmness and viscosity of the yoghurt as the concentration of turmeric increased. Taking into consideration the values from **Table 2**, the most appropriate percentage of spice juice to be incorporated into a yoghurt product would be between 1-4%.

Table 2. Recommended daily dosages of some spices.

Form	Garlic Amount	Ginger Amount	Onion Amount	Turmeric Amount
Fresh raw	~2-4gb	~2-4gb	~50gc	~1.5-3gb
Dried powder	~900mga	~1gb	~20gc	~1-3gb
Essential oil	~0.09-0.36mLb	(no info found)	(no info found)	(no info found)
Extract (solid)	~600-1,200mg	(no info found)	(no info found)	~0.4gb
Juice		~1.5-3mLb	~50mLc	~4mLb

Source: Amagase et al. [73], University of Maryland-Medical Center 2011 [74], Blumenthal et al. [75].

EXPECTED FUTURE TRENDS IN YOGHURT SALES

Globally, consumers spend \$73 billion per year on yoghurt [73-76]. Yoghurt is growing fast, at 7% annually, and is among the top five foods for sales growth. The U.S. market for yoghurt is estimated to be worth \$6 billion [77]. In 2009, United States was the second largest market for functional foods, whereas Japan was the first. The functional foods market is expected to increase from \$7.1 billion and the yoghurt market increased from \$9.7 billion in 2005 to \$15.4 billion in 2010 [78]. Highly condensed Yoghurt can be made available in different forms such as drinking yoghurt, lactose-hydrolyzed yoghurt, strained yoghurt, frozen yoghurt (with categories soft, hard, or mousse), dried yoghurt, bio-yoghurt (yoghurt made with different live cultures other than the 2 most widely used ones), vegetable oil yoghurt, soy yoghurt, and chemically acidified yoghurt [15] and yoghurt has always been successful in making people enjoy the products. Ever-growing consumer demand for convenience, combined with a wholesome diet and preference for natural ingredients, has led to growth in the functional beverage market. Current trends and changing consumer needs indicate a great opportunity for innovations and developments in fermented milks. While scientific and clinical evidence is mounting to corroborate the consumer perception of health from fermented milks, the probiotics, prebiotics, synbiotics, and associated ingredients are able to add an attractive dimension to cultured dairy products. The compounds responsible for yoghurt aroma can be used as additives. Within the prescribed range, 1-nonen-3-one can be added to food, pharmaceutical, cosmetic, and perfume compositions to flavor the products and to impart aroma, particularly to dairy products and coffee extract. The 1-nonen-3-one can also be combined with other compounds that provide taste and aroma, and the combined compounds can be added to the compositions [79]. The application of yoghurt to prepare baked goods such as bread has been patented, which are reported to have improved flavor and other properties that can be obtained by incorporating yoghurt in the dough [80]. Owing to expanding market size of dairy companies, there has been merging of dairy products and fruit beverage markets with the introduction of “juice ceuticals” such as fruit-yoghurt beverages that are typical examples of hybrid dairy products which are able to offer health, flavor, and convenience at the same time. Another potential growth area for fermented milks includes value-added products such as low-calorie, reduced-fat varieties, and those fortified with physiologically active ingredients, including fibers, phyto-sterols, omega-3-fatty acids, whey-based ingredients, antioxidant vitamins, and iso-flavones that provide specific health benefits beyond basic nutrition. Continuous efforts are being made to develop yoghurt containing certain nonconventional food sources such as soybeans and millet and convert them to more acceptable and palatable forms thus producing low-cost, nutritious fermented foods. Use of bio-preservatives and certain innovative technologies such as membrane processing, high-pressure processing, and carbonation can lead to milk fermentation under predictable, controllable, and precise conditions to yield hygienic fermented milks of high nutritive value [81], which can help to further improve the industry and add value [82-84].

CONCLUSION

Yoghurt has been a part of human diet in many parts of the world because of acceptance of its taste (along with remarkable beneficial effects). With the advancement of technology, it is now common to find different types of flavors such as peach, red fruits, lemon, apple, and so forth. Aroma additions provide various flavor possibilities in the dairy market that has increased the popularity of products such as yoghurt, milk drinks, desserts, and others. To satisfy consumer demands, manufacturers increasingly yoghurts with reduced fat content replaced by thickeners and gelling agents and can lead to change in the distribution of flavor molecules. Yoghurt is a major source of nutrients for both vegetarians and non-vegetarians also be effective in cytokine production, T-cell function and natural killer-cell activity, and thereby result an overall immunological enhancement [4].

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