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Functional Foods in Health and Disease

Use of probiotics for nutritional enrichment of dairy products

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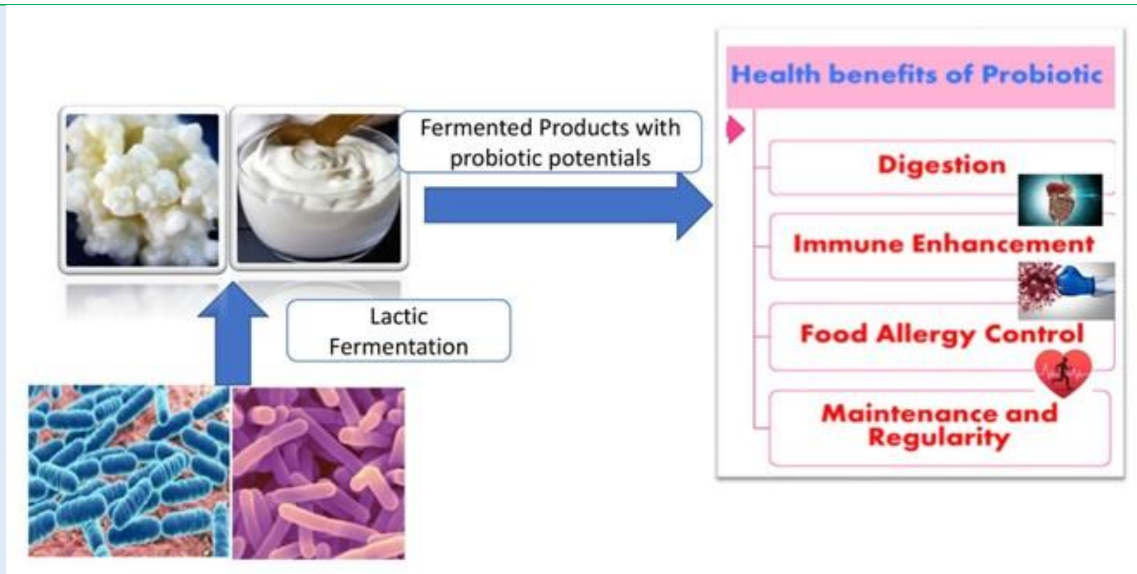
ABSTRACT

Several food products, prepared with the help of beneficial microbes, can fit into the current definition of probiotics which have been consumed by mankind since ancient times. Beneficial microbes are the agents that produce many fermented foods and beverages, which are popular in different parts of the world. Indications about the use of products with healthy microbes are given in *Vedic* literature and are also depicted in ancient Egyptian and European treatises. However, systematic studies on probiotics started after the publication of the book, *Prolongation of life*, by Eli Metchnikoff in 1907. During the last century, the use of probiotics as food and pharma products gained substantial rise. This review intends to compile and analyze the literature pertaining to the use of probiotics as food products, especially for nutritional enrichment. The review will focus on the key issues important to establishing the requirement to re-assure the efficacy and safety after strain development, process standardization, product formulations, bioavailability of the live cells and biomolecules, and the effect of processing, etc. Current literatures addressing the mechanisms of action for probiotic function and the development of novel probiotic foods with health claims and meeting the nutritional requirement through fermentation are necessary to better understand the product and its application.

KEYWORDS: Probiotics, prebiotics, nutritional enrichment, fermented foods, bioavailability, efficacy, regulatory, safety, synbiotic, functional food



INTRODUCTION One of the key challenges faced in the 21st century is the need to feed a rapidly increasing human population through a balanced diet, despite increasingly limited natural resources. It is estimated that roughly 1 out of 9 people in the world are undernourished, mainly due to protein-energy malnutrition [1]. The role of balanced nutrition in regard to the maintenance of health is of much interest to the scientific community, as well as the efficacy of some foods in reducing the risk of various diseases, including certain metabolic syndromes, has been proven through numerous studies.

Consequently, there has been an increase in research to find out new natural components and the development of novel foods, enabling innovation in the food area and the creation of new market niches mainly related to functional products. One's diet not only satisfies satiety, but also acts to support the metabolic needs of consumers by providing sufficient levels of nutrients, vitamins, and minerals. Additionally, recent nutritional advancements have demonstrated that diet also plays an important role in the prevention and possible cure of various diseases by regulating multiple physiological functions.

Probiotics are one of the most consumed food supplements and they constitute relentlessly burgeoning a billion-dollar industry worldwide. The word probiotics comes from the Latin word *Pro* ("for") and the Greek word, *Bios* ("life"). Metchnikoff is considered to be the "Father of Probiotics", but there are numerous scientists who have individually been credited with coining the word itself: *viz*., Kollath (1953), Lilly and Stillwell (1965), Parker (1974), Fuller (1989), and others [3-6]. They each had their own definition of the term. The World Health Organization/Food Agricultural Organization (WHO/FAO) developed a widely accepted definition in 2001 [2], which was slightly modified by The International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2014 as the following: "Probiotics are live microorganisms, that, when administered in adequate amounts, confer a health benefit on the host" [8]. However, for Indians, Probiotics and Synbiotics are not new concepts, as we have evidence of a product called as *Panchamrut,* which is in vogue since *Vedic* times and used in every ritual. This has concept of using curd, which has healthy Bacteria and adds honey, which is a good source of Prebiotics. However, the credit goes to Eli Metchikoff and Prof. Shirota of Japan for disseminating the knowledge of Probiotic science today.

Probiotics have been supplemented in various food products, such as cheese, ice cream, yogurt, nutrition bars, numerous snacks, breakfast cereals, fruit juices and infant formulas. Additionally, they are also commercialized as pharma products as Lyophilized Capsules, Tablets, etc. Physicians, specifically Gastroenterologists, tend to largely support the consumption of Probiotics, worldwide [3]. In recent years, fermented milks (which are among Fermented Foods) have been largely promoted through media due to their proven nutritional and health benefits. Fermented milks and dairy products provide the best matrices for Probiotics and are subject to multidimensional studies for their functional attributes [4]. Furthermore, the consumption of fermented milks has been quickly increasing worldwide, as many consumers recognize the health benefits associated with probiotic fermented milks [5-6]. A special group of bacteria, Lactic Acid Bacteria (e.g., *Lactobacilli, Streptococci* and *Bifidobacteria*), hold the main Probiotic preparations currently available in the market. These bacteria produce Lactic Acid, a major metabolic product that is an important constituent of the human gastrointestinal microflora. These probiotics (which are used in foods) are primarily added as part of the fermentation process. However, they are now being increasingly added as supplements as well. Furthermore, there is also an increasing trend in using probiotics as Nutraceuticals, being available in various formats. However, pure cultures can be used in addition to foods.

The number of species and the abundance of microorganisms added in food can vary from a single species to a consortium. Most commercialized probiotic foods are dairy-based, as it provides an excellent carrier for probiotic bacteria, specifically for *Lactobacillus casei*, *Lactobacillus acidophilus* and *Bifidobacterium* species [7-8]. Dairy-based probiotic foods have a naturally supportive matrix; However, for non-dairy-based probiotic foods, it is much more complicated, as each food has its own unique matrix which makes it more difficult for food scientists to design a stable and cost-effective non-dairy probiotic food that maintains high sensory and nutritional properties. Despite many technical difficulties, numerous non-dairy probiotic foods have reached supermarket shelves worldwide. A few non-dairy probiotic foods, such as fermented cereals, meat products (sausages, hams, beef), beverages (alcohol-free beer), fruits and vegetables (snacks, juices, etc) have recently drawn attention for consumers and those in the scientific community as well [9].

Probiotic strains have a high acid and bile tolerance, which contributes to their ability to survive passage through the GI tract. These are intrinsic characteristics of the strain, which can be improved by the protective action of carrier foods and/or by the presence of nutrients, such as Metabolizable Sugars. Dairy Products are the most common food matrices used as vehicles for probiotics because they can enhance the transit tolerance of Bacteria. For example, a couple strains of Lactobacillus and Bifidobacterium have been shown to better tolerate acidic stress when ingested with milk products [10]. The majority of commercially available cultures are preserved as Direct Vat Set (DVS) which are commonly preferred, as it is hard to propagate Probiotic cells in an industrial fabrication site. These DVS cultures are then applied either as Freeze-Dried Powders or as deep-frozen cultures that are highly concentrated. The deep-frozen cultures comprise a microbial load of more than 10^{10} colony forming unit (CFU) /g, while freeze-dried culture carries more than 10^{11} CFU/g. The cell concentration per gram of product depends on the choice of culture and the type of organism. Therefore, specific information regarding strain properties must be examined in advance for process optimization [11].

The ability of Probiotic strains to survive passage through the GI tract can be mainly attributed to their acid and bile tolerance. These are intrinsic characteristics of the strain, which can be improved by the protective action of carrier foods and/or by the presence of nutrients such as Metabolizable Sugars. The most common food matrices used as Probiotic vehicles are Dairy Products, which can enhance the transit tolerance of Bacteria. Some strains of Lactobacillus and Bifidobacterium have been shown to tolerate acidic stress when ingested with milk products. Most commercially available cultures are preserved as Direct Vat Set (DVS), applied either as freeze-dried powders or as highly concentrated deep-frozen cultures. Among these, DVS cultures are commonly preferred as it is hard to propagate Probiotic cells in an industrial fabrication site. These probiotic cultures are incorporated to enhance the bioavailability of biomolecules present in fermented milks, like short chain fatty acids, bioactive peptides, organic acids, antimicrobial substances and enhances the immunity and digestibility of protein, fat and carbohydrates.

Probiotic products are becoming important to society because of its health benefits and are important to the food industry because of premium income. Hence, the market for the same is increasing. A report by Global Market Insights Inc. indicates that the probiotics market will be worth 4.15 billion USD by 2027 and its growth is expected to register 9% Compound Annual Growth Rate (CAGR) between 2021 to 2027 [12]. The Indian Probiotic market is expected to grow at the rate of 20% per annum, doubling in the next 5 years. The Indian market for food ingredient probiotics is largely import driven. The research on this subject that has been done in India has been compiled and published as a status paper by the Probiotic Association of India [13].

The present review is aimed at bringing out the Nutritional and Functional attributes of probiotic food products so that the stakeholders from government, academia and industry can focus on their benefits and that can be conveyed to the society in an effective way.

Products Manufactured with Probiotics as Sole Culture: Acidophilus and Bifidus were the most popular cultures, therefore the original probiotic products prepared were employing these cultures alone or in combination. A few products are briefly described below.

Acidophilus Products: Acidophilus products are cultured Milk products made by fermentation with *Lb. acidophilus* alone or in combination with some other bacteria or yeasts [14]. The remarkable thing is that the culture *Lb. acidophilus* is a natural inhabitant of the intestinal tract of humans and animals and can establish itself there [15]. This property helps in combating intestinal pathogens and cures several intestinal disorders. Additionally, it gives several other health benefits and has become very popular. This culture was one of the oldest candidates as a probiotic and now it is incorporated in several foods and feeds for the improvement of growth and performance of the host. Traditional Acidophilus milk is prepared by inoculating *Lb. acidophilus* culture at the rate of 2-5% in autoclaved or severally boiled milk. The milk is incubated at 37ºC, till it attains 1 to 1.5% acidity. This milk gives a cooked flavor and an unappetizing, flat taste. Hence, it failed to become popular and was consumed as medicine only when needed. Considering the limitation of traditional Acidophilus milk, attempts were made to prepare several other products which can supply large numbers of viable Acidophilus cells in acceptable forms. These attempts were directed towards the supplementation of *Lb. acidophilus* with other flavor producing cultures, concentration and drying of the products or dispensing cell concentrates into other popular dairy products, like pasteurized milk or ice cream. Table 1 shows the list of products prepared using Acidophilus, as well as Bifidus cultures. The recommended dose of live culture consumption is 10^8 cells per gram to derive maximum benefits [16]. Hence, the required quantity of the product needs to be consumed to have the recommended dose.

Table 1. Acidophilus-Bifidus Products

	Name of the Product	**Organisms Involved**	**Principle of Processing**
1.	Acidophilus Sour Milk	*Lb. acidophilus*	Fermentation
2.	A-38	*Lc. lactis* + *Leuconostoc* spp. + *Lb. acidophilus*	Both Groups are Fermented Separately and Mixed
3.	ACO-Yoghurt	Yoghurt culture + *Lb. acidophilus*	Both Groups are Fermented Separately and Mixed
4.	Acidophilus Yoghurt	Yoghurt culture + *Lb. acidophilus*	Fermentation
5.	Acidophilus Bifidus Yoghurt	Yoghurt culture + *Lb. acidophilus* + *B. bifidum*	Fermentation
6.	Bioghurt	*S. thermophilus* + *Lb. acidophilus*	Fermentation
7.	Bifighurt	*S. thermophilus* + *B. bifidum*	Fermentation
8.	Biograde	*S. thermophilus* + *B. bifidum* + *Lb. acidophilus*	Fermentation
9.	Sweet Acidophilus Milk	*Lb. acidophilus*	Non-Fermented added in Chilled Pasteurized Milk
10.	Acidophilus Yeast Milk	*Lb. acidophilus* + lactose fermenting yeast	Acid & Alcohol Fermentation
11.	Acidophilin	*Lb. acidophilus* + *Lc. lactis* + yeast	Mixed Acid & Alcohol Fermentation
12.	Acidophilus Ice-Cream	*Lb. acidophilus*	Ice-Cream added with Concentrated *Lb. acidophilus* before Freezing
13.	Acidophilus Paste	*Lb. acidophilus*	Concentrated after Fermentation by Centrifugation
14.	Dried Acidophilus (Powder, Tablets, Capsules)	*Lb. acidophilus*	Dried Concentrated Cells in Milk Based Media by Spray Drying, Vacuum Drying or Freeze Drying
15.	Acidophilus Whey	*Lb. acidophilus*	Fermented Whey

Acidophilus Milk has a high biological value, due to the availability of essential Amino Acids of milk proteins and microbial cell protein. Its functionality depends on the type of milk used and on the manufacturing process employed. Folic acid, riboflavin, thiamine, niacin, pyridoxine, and vitamin k, (all of which are slowly absorbed by the body) have all reportedly been shown to be synthesized by *Lactobacillus acidophilus*. The vitamins of the B-complex are commonly attained as natural ingredients in foods, so the addition of *L. acidophilus* to the diet will be more effective in meeting those requirements. The bio-availabilities of minerals, such as copper, zinc, manganese, calcium, iron and phosphorus may also be increased by consuming fermented dairy products and the digestion of proteins improved as well [18].

During manufacturing, the denaturation of milk serum proteins and the release of peptides that are essential for the growth of *Lb. acidophilus* can be achieved through high heat treatments. Different flavor compounds may also be added to Acidophilus Milk during manufacturing to improve its overall taste, as well as sensory acceptability. Acidophilus Milk is known to have a slightly thick texture with a distinct tangy flavor. Shiby & Mishra investigated the effects of A-38 on Plasma Triacylglycerol (TAG) in human subjects, as well as glucose and insulin concentrations. The addition of green banana pulp into fermented milk was tested as well [19]. This was found to cause a more rapid decrease in pH. However, the protein, carbohydrate, lipid, and fiber levels did not change, which indicates that there was no ultimate change in calorie value [20].

Apart from the milk-based Acidophilus products, it has been recently suggested that *L. acidophilus* fermented pear juice could act as a new strategy for anti-hyperglycemia and antihypertensive therapy, which could act to reduce the oxidative stress linked with Type 2 Diabetes [21]. A new Probiotic Product, called Oblea (a traditional wafer dehydrated Mexican dessert) was developed using sweet goat whey fermented with *B. infantis* or *L. acidophilus* and proved to maintain concentrations above the minimum amount required in a probiotic product [22]. In a functional bread, combining the starch-based coatings (dispersed or multilayer) and the microencapsulation showed that *L. acidophilus* survived after baking and storage time, although reduction was higher in the sandwich treatment (Starch Solution/Sprayed Microcapsules/Starch Solution) [23].

Bifidus Products: Bifidiobacteria are normal inhabitants of the intestinal tract of newborns and infants. They possess special health properties and increase the resistance of the infant to several disorders. The milk cultured with Bifidobacteria is called Bifidus milk. The Bifidus milk has its origin in Germany and is produced in small quantities in some of the European countries. Human strains of *Bifidobacterium bifidum* or *Bifidobacterium longum* is used as culture. The coagulated product contains 10^8-10^9/ml of viable Bifidobacteria and has a pH range of 4.3 to 4.7. Bifidus milk can be produced as a stirred product, which is easily digestible than the milk that it's made from. It has been used to protect against gut microflora imbalances in the treatment of chronic constipation, liver diseases, and as an aid in the treatment of gastrointestinal disorders [24]. True Bifidobacteria are strict anaerobes and slow growers in Milk, hence it is difficult to prepare Bifidus Milk. However, the product is made from severely heat-treated milk taking greater aseptic precautions and using high rates of inoculum. Protein enrichment and fat standardization are common practices of Bifidus milk production, creating a slightly acidic flavor and a characteristic aroma with a Lactic Acid to Acetic Acid ratio of 2:3 [25]. The species regularly employed are *B. bifidum or B. longgum.* However, the use of aerotolerant strains of *B. adolescentis* have been found to give better product. Several Humanized Milks or Baby Foods have also been developed by making use of Bifidobacteria or *Lb. acidophilus.* In the dairy industry, probiotic strains of *Bifidobacterium* are used in development of humanized milks or baby foods, and they are also used as a component of the starter culture intended to produce fermented beverages, cottage cheeses, ice creams, etc. [26]. Bifidus milk supports the treatments of gastrointestinal disorders and liver diseases and is digested easily [27].

Products made with Probiotics as Secondary Culture: Several popular products are prepared by primary start cultures and added with different types of probiotic bacteria as supplementary cultures. Yogurt is the best example of such products, which is available worldwide.

Yogurts: Various countries produce a wide range of fermented milk products, but the most common example is yogurt, which is manufactured as either Set-, Stirred- and/or Drinking-Types. Additionally, the products are usually flavored by adding fruit preparations and coloring matter [28]. Classic yogurt is prepared by fermenting heat treated Milk with the help of a Symbiotic culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* [29]. Overall, the manufacturing process of probiotic yogurt and classical yogurt is quite similar, but the fermentation time of natural probiotic yogurt is slightly longer and the product is said to have a slightly sweeter taste compared to classic yogurt. Yogurts have increasingly been prepared with probiotic microorganisms with differing viability over a range of shelf lives. Numerous scientific investigations have been carried out to increase the viability and functionality of probiotic strains in yogurt preparations. The most common probiotic cultures added to yogurt are selected strains of Lactobacilli and Bifidobacteria [30]. However, the survival of Lactobacillus and Bifidobacterium spp. in yogurt may be affected by several factors, such as strains of probiotic bacteria, concentration of metabolites (Acetic Acids and Lactic Acid), presence of hydrogen peroxide and dissolved oxygen, buffering capacity of the media, pH, and storage temperature. The viability of probiotics in yogurt depends on factors like the availability of various nutrients, concentration of solutes, inoculation level, incubation temperature, growth promoters and inhibitors, storage temperature, and fermentation time. Yogurt contains about 0.8% to 1.2% Lactic Acid and has a shelf-life of 3-4 weeks at refrigeration temperature. Supplementation with different substances has resulted in varying effects on the viability of probiotic bacteria in yogurt. However, yogurt was used in regard to the history of fermentation, due to production of bioactive molecules which may be investigated in the treatment of microbes as bioactive molecules targeted to manage specific pathologies [31]. One of the possible mechanisms that may help to improve the viability of probiotic bacteria is the oxygen scavenging effect of ascorbic acid. The addition of whey proteins may enhance the viability of some probiotic bacteria as well, due to their buffering capacity, especially in yogurts with added fruit pulp. The incorporation of prebiotics, like fructooligosaccharides and inulin [32], nutraceutical's containing isoflavones, phytosterols, and omega-3-fatty acids [33] in yogurt formulations stimulated the viability and activity of probiotic bacteria. Also, the addition of cysteine to yogurt at 250 and 500 mg/L was proven to be associated with higher viability of *L. acidophilus* during manufacturing and storage, although the viability of bifidobacteria was adversely affected by similar levels in different starter cultures. However, in mixed culture products, symbiotic and antagonistic interactions between probiotic and standard starter cultures are very important, as well as those between different probiotic cultures. The probiotic cultures must be compatible with each other the standard starter cultures since these microorganisms could produce inhibitory substances and affect viability of probiotic bacteria [34].

Kefir: Kefir is a foamy, effervescent milk product resulting from mixed Lactic Acid and Alcoholic Fermentation of milk by kefir grains. It is an old and historic product from the Caucasian mountains in Russia. Kefir grains are gelatinous, white or cream-colored, irregular grains of varying size (0.5 – 2.0 cm diameter), added with Wheat grain or Walnut [35]. They are made of polysaccharides called "Kefiran" and are insoluble in water. Within the folds of granules, Bacteria and Yeasts reside in a symbiotic relationship. It is found that Kefir grains contain at least six functionally different groups of organisms: viz., (i) Mesophilic Homofermentative Lactic Streptococci, (ii) Mesophilic Heterofermentative Streptococci, (ii) Thermophilic Lactobacilli, (iv) Mesophilic Lactobacilli (v) Yeasts, and (vi) Acetic Acid Bacteria. All these organisms grow in association during Kefir manufacture and produce Lactic Acid (0.91 -1%), and Alcohol (0.5 -1.0%) and CO_2 (0.03 – 0.07%) as major end products [36].

Kefir is expected to contain several potentially probiotic organisms and it has been found to show health benefits. It improves digestion, prevents constipation, and regulates bowel movement. It is a natural antioxidant that helps to keep skin youthful and glowing. Kefir can enhance brain functioning and aids in reducing stress. The regular use of Kefir helps to reduce high cholesterol levels. Similarly, Kefir is found to be effective for heart health, as well as the respiratory and immune systems. Kefir has been associated with cholesterol metabolism, Angiotensin-Converting Enzyme (ACE) inhibition, increased speed of wound healing, tumor suppression, antimicrobial activity, and the modulation of the immune system, including the alleviation of allergies and asthma. These reports have led to increased interest in Kefir as a focus of research and as a potential Probiotic-containing product [37].

Koumiss: Koumiss is a product similar to Kefir that is made by acid and alcoholic fermentation of milk. It is very popular in Russia and Central Asia and is traditionally made from Mare's Milk. However, due to an increased demand of Koumiss and a short supply of Mare's Milk, the product is now made from cow's milk after a few modifications. Koumiss culture consists of *Lb. delbrueckii* subsp*. bulgaricus, Lb. acidophilus* and *Kluyveromyces lactis or K. marxianus* subsp *marxianus.* Yeast and Lactobacili grow in association in this product and produce 1 to 1.5% Lactic Acid, 1-2% Alcohol and 0.5-0.9% Carbon Dioxide. The Koumiss is graded as weak, medium and strong based on acidity and alcohol contents. In Russia, Koumiss is used for the treatment of Pulmonary Tuberculosis. Zha mentioned that Koumiss was used to cure Tuberculosis effectively during every summer and autumn at the Ximeng Mongolian Medical Research Institute. It was proved that the causative agent of Tuberculosis (*Mycobacterium tuberculosis*) cannot survive in the Mare Milk because of the Antituberculosis element generated by the microflora of Koumiss [38]. When Koumiss is used in clinical practice to treat Tuberculosis, a 60%–91% rate of recovery was reported, which was confirmed by lab techniques such as X-rays and the Tuberculin test. The disappearance of the symptoms is also an indication of effective treatment [39]. Yogurt and kefir are fermented milk products that originated in Middle Eastern countries. The sour milk is stored in porous earthenware or hung in cloth bags to allow its concentration. In some areas, it is rolled into balls and sun dried. The product contains mixed microflora consisting of *Lc. Lactis, S. thermophilus, Lb. bulgaricus, Lb. plantarum, Lb.brevis, Lb. casei* and yeast.

Probiotic Buttermilk: True buttermilk is the fluid remaining after cream is churned into butter. However, more commonly the cultured Buttermilk is produced by souring skim milk with Mesophilic Lactococci and Leuconostocs [40]. They produce a mild sour taste, with acidity generally around 0.8 to 0.9% and give a typical Diacetyl flavor. Generally, Mesophilic Mixed Starter Cultures comprise of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *Cremoris*, *Lactococcus lactis* subsp. *Lactis* Biovar. *Diacetylactis* and *Leuconostoc mesenteroides* subsp. *Cremoris* are used. There are certain slimy and/or rope-like Lactic strains that are also used to improve product consistency. Milk may also be supplemented with 0.1-0.2% citrate to impart high flavor intensity in buttermilk. Agitation and cooling also affect the flavor of the product.

Lassi (a refreshing, drinkable yoghurt-like beverage popular in the Indian Subcontinent) is prepared from milk with 1.5 to 4.5% fat from Dahi, followed by vigorous stirring to break the curd and an addition of sugar syrup and an additional flavor [41]. It is a creamy-white, viscous liquid with a sweet, rich aroma and is mild to acidic in taste. Starter culture used in the manufacturing of Lassi is similar to those that are used in Dahi.

There is plenty of completed research on Probiotic Lassi or *Chaach* or Buttermilk in India. In table 2, some of the products developed at the Faculty of Dairy Science, Anand Agricultural University Anand are shown. [42].

Table 2. Probiotic and Synbiotic Products Developed at Anand

Product	Ingredients	Remarks
Synbiotic Dahi	Milk, Inulin, Sugar	Set Coagulated Product with 10^8 Viable Cells of Probiotic Lactobacilli per gram.
Synbiotic Raita	Milk, Inulin, Fructooligosachharide, Tomato, Cucumber, Onion, Banana, Sapota, Sugar	Stirred Yogurt Type Products Fermented by Probiotic Lactobacilli and Garnished with Fruits and Vegetables.
Synbiotic Lassi	Milk, Oat, FOS, Carrot, Mango, Sugar, Honey	Thick Liquid with Probiotics and Shelf Life of 3 weeks at 5°C.
Whey Drink	Whey, Sugar, Pineapple	Beverage with Fruit Pieces and 10^8 cells/ml of Probiotic Lactobacilli.
Herbal Probiotic Lassi	Milk, Safed Musli, Sugar, Honey	Milk Fermented by Probiotic Lactobacilli and Supplemented with Herbs.
Protein Rich Lassi	Milk, Spirulina, Sugar	Fermented Milk Enriched in Protein by Spirulina.
Acidophilus Banana Powder	Acidophilus Milk, Banana, Sugar, Elachi	Dried Product with 10 million/g Viable Cells of *Lb. acidophilus*.
Acidophilus Wheat Malt Powder	Acidophilus Milk, Wheat Malt, Sugar, Cocoa Powder	Dried Product with 10 million/g Viable Cells of *Lb. acidophilus*.
Milk-Rice Probiotic Food	milk, Rice, Freeze Dried Probiotic Culture	Milk and Rice were Fermented and Spray Dried and Blended with Freeze Dried Probiotic Lactobacillus Cells.
Probiotic Carbonated Beverage	milk, Sugar	Milk Fermented by Probiotic Lactobacilli and Supplemented with Artificial Carbonation
Carbonated Probiotic Beverage Employing Yeast	Paneer Whey, Sugar	Whey Fermented with *L.helveticus* and *K.marxianus* @ 2 % v/v)
Probiotic Ragi Ice Cream	Probiotic Culture, Finger Millet, milk Solids	Ice-Cream Supplemented with Finger Millets (Ragi) and added with Indigenous Probiotic Culture
Probiotic Oat Based Lassi	Probiotic Culture, Oat, Whey Protein Concentrate	An Oat based Fermented Beverage using Oat Bran, Cow Milk and Probiotic Bacteria
Probiotic Smoothie	Probiotic Culture, SMP, WPI, Oat, Sugar, Mango Pulp	Smoothie was Prepared using Dry Dairy Ingredients

Probiotic Cheese: As a probiotic food carrier, cheese is one of the most versatile food products available today, which creates opportunities for different marketing strategies. However, the development of probiotic cheeses requires knowledge of all processing steps, as well as how they positively or negatively influence the survival of the contained microorganisms during shelf life. In comparison to yogurt and fermented milks, cheese holds multiple advantages as a probiotic carrier. It has a higher pH and therefore buffering capacity [43], high energy, higher fat content, is nutritious and can survive a longer shelf life while maintaining higher viability, which has been demonstrated through multiple studies. The probiotics in cheese were found to survive the passage through the simulated human gastrointestinal tract and significantly increase the numbers of probiotic cells in the gut. However, when looking at the serving size of both cheese and yogurt, cheese requires more probiotic cells and higher viability to result in similar health benefits. Cheese was first introduced to the probiotic industry in 2006, when a company decided to test the survival and growth of probiotic strains in cheese [44]. At that time, there were only a few probiotic cheese products were available in the market. This original test showed that less than 10% of the bacteria were lost. Commercial probiotic cheese was first developed by the Mills DA, in Oslo, Norway. Probiotic cheeses can be found in over 200 different forms, including as fresh, hard, semi-hard, etc in various markets. In comparison to yogurt, semi-hard and hard cheese, have a relatively low recommended daily intake and need a slightly higher inoculation level of probiotics (about four to five times). On the other hand, fresh cheese has a high recommended daily intake, with a limited shelf life at refrigeration temperature, meaning it could serve as a high potential food for a carrier for probiotics [45]. Milk fermented with with *L. acidispiscis*, *L. pentosus* and mixed i.e combination of these two cultures have shown high ACE-Inhibitory activity. The *L. plantarum* and mixed fermentations have shown higher iron-binding activity, whereas the *L. acidipiscis* and mixed fermentations had the highest calcium-binding activity. This demonstrates that probiotic microorganisms isolated from double cream cheese have great potential to be used in the production of functional foods [46]. Mozzarella cheese is a popular member of the Pasta Filata Cheeses, which are renowned for their ability to be molded, shredded, melted or stretched. These properties are all due to distinctive plasticizing and texturing treatments of curds in hot water. Songisepp *et al*., developed an original probiotic cheese based on the “Pikantne”, an Estonian semisoft cheese. The cheese was produced by two methods using cheese starter cultures (Probat 505) in combination with 0.04% of Probiotic *Lactobacillus fermentum* strain ME-3 (10^9 CFU/mL) with high antimicrobial activity and antioxidative properties. The metabolic functions of humans can be influenced by Lactobacilli in the gut. For example, in Russian adults with obesity and hypertension, symptoms of metabolic syndrome were reduced by the probiotic cheese prepared with *Lactobacillus plantarum* TENSIA (Deutsche Sammlung Für Mikroorganismen, DSM 21380) [47]. Encapsulated *B. infantis, B. bifidum,* and *Bifidobacterium longum* have been used in the manufacturing of cheeses, like Crescenza cheese, and may be used to improve viability and protect various probiotic organisms [48]. For semi-hard and hard cheeses, the addition of a dried culture during salting of curd is used as a method of introduction, with some enzyme preparations involved as well. This method minimizes the losses of bacterial cells to whey and eliminates the effects of competition with lactic acid bacteria during milk ripening.

Use of prebiotics as a means of nutritional enrichment: In 2008, Dietary Prebiotics were defined as “a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” by the 6th Meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) [49].

Thus, an integration of synergistically acting probiotics and prebiotics is defined in terms of "Synbiotic." Synbiotics' modes of action are directly linked with the type of live culture being used and they offer health benefits in two ways: (1) by improving the viability of the probiotic cells and ensuring that they reach the colon in satisfactory populations to give a beneficial health effect and (2) with prebiotics, probiotic cells can survive and increase in numbers. A true probiotic is especially vulnerable to variations in temperature, pH, and oxygen levels in the digestive system, and cannot survive well without the presence of certain prebiotics. In other words, the prebiotics are basically supportive and preservative agents for the live cultures [50].

Synbiotics are usually prepared by adding specific probiotics, as well as prebiotic ingredients or value-added ingredients (Table 3). As of April 1, 2011, under Food for Specific Health Use, more than 350 products have been approved with a claim to control GI functions (maintaining good GI conditions and bowel movements).

This list includes oligosaccharides, nondigestible oligosaccharides/dietary fibers, *Lactobacillus,* and *Bifidobacteria* as well. Additionally, preparations with cassava starch or inulin, milk and three different LAB; Lactulose, Inulin with *L. acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12; inulin, FOS and Honey with *L. acidophilus*; Sugar with inulin and probiotics; Raftiline, Raftilose with Honey, Milk, and LAB are worth mentioning [50].

Table 3: Examples of Prebiotics and Synbiotics used in Human Nutrition [51]

Prebiotics	Probiotic Organism
FOS GOS Inulin XOS Lactitol Lactosucrose Lactulose Soy Oligosaccharides TOS	*Lactobacillus* genus bacteria *Lactobacillus, Streptococcus* and *Bifidobacterium* genus bacteria + FOS *Lactobacillus*, *Bifidobacterium*, *Enterococcus* genus bacteria + FOS *Lactobacillus* and *Bifidobacterium* genus bacteria + oligofructose *Lactobacillus* and *Bifidobacterium* genus bacteria + inulin

Functional Effects of Prebiotics on Human Health: Poor eating habits have had a prominent effect on human health and overall quality of life, especially in recent years. The overconsumption of fats (specifically saturated), sugars, as well as salt and sodium, mixed with the decreasing consumption of dietary fibers, has led to an overall rise in the prevalence of chronic diseases, including cardiovascular diseases, obesity, diabetes, and cancer [52]. Prebiotics are the functional compounds of foods, which play an important role in the prevention and treatment of gastrointestinal diseases. Due to Prebiotics' selective metabolism in the intestinal tract, their consumption may result in multiple advantages for the host [53]. Healthy microbiota can produce metabolites that interact with the immune system and aid in the reduction or prevention of intestinal inflammatory disease [54].

The food industry is adding value to products by supplementing them with prebiotics, which include healthy benefits to host nutrition, inhibiting the growth of pathogens, and promoting beneficial microbiota [55] (Table 4). These microbiotas can cause fermentation of nondigestible fibers, promote the synthesis of vitamins B and K, promote metabolism of plant compounds, improve energy conservation and production of scfa and polyamines, improve gastrointestinal motility and function, cholesterol reduction, and stimulation of the local immune system [57].

The regular intake of prebiotic fructans, such as the FOS and Polysaccharides (e.g., Inulin), can prove to be beneficial in a variety of ways. They act to sustain health and overall well-being by (i) improving blood parameters, (ii) enhancing resistance against intestinal and extra-intestinal pathogens, (iii) modulating immune responses, and (iv) decreasing allergic responses [58].

The proximal part of the Colon is where Inulin and Fructo Oligo Saccharides (FOS) are thought to exert their beneficial activities, but there is a great interest in the distal colon, where many diseases originate and finding different prebiotics that can exert their respective health benefits there as well. Arabinogalactans and Branched Fructans (e.g., Cereal Graminans) are thought to be beneficial in this regard [59], due to their indirect mechanisms that have a positive influence on intestinal microbiota

Table 4: Use of Synbiotics in Product Development

Products	Probiotic	Prebiotic	Use of Prebiotic and Their Effect in Synbiotic Products	References
Synbiotic Semi-Hard Cheese	*Lb. paracasei* INIA P272	Fructo-Oligosaccharides (FOS)	Increasing the Probiotic Strain Viability in the Host. Organic Acid Detection and Volatile Fraction Analysis of the Cheese Revealed a Stimulation of *Lb. paracasei* Metabolism when FOS was Present.	[60]
Synbiotic Yogurt	*Lb. rhamnosus* and *Lb. reuteri*	Inulin, Lactulose, and Oligofructose	Inulin and Lactulose showed the Lowest Syneresis Inulin - *Lactobacillus rhamnosus* and *Lactobacillus reuteri*, showed the Best Viability and Provides a Microbial Balance in the Gut. Inulin and Lactulose - showed the Most Desirable Sourness	[61]
Synbiotic Ice Cream	*Saccharomyces boulardii* CNCM I-745	Inulin	Addition of Inulin Increased the Viability of *S. boulardii* CNCM I-745 with Viable Count of 6.16 log CFU/g in the Synbiotic ice cream after 120 d of Storage. Improved Firmness and Melting Property and Stability. About 27 Volatile Compounds Identified in Different Ice Cream Formulations and their Concentration was the Highest (3983 µg/L) in Synbiotic Ice Cream as Compared to Control (616.4 µg/L).	[62]
Synbiotic Lassi	*Str. thermophilus* MTCC 5460	Honey (at 5% level) Honey Contains 4-5% Fructo-Oligosaccharides which may Serve as Prebiotic Agents	Slightly Higher *Lactobacilli* and *Streptococci* Counts and a Significantly ($P<0.05$) Higher Sensory Scores.	[63]
Synbiotic Yoghurt	*L. rhamnosus*	Fibre Source (0.5 % oat flour)	Good Storage Stability The Viability of Probiotics was Good in Synbiotic Yogurt Made with both the Probiotic (*L. rhamnosus*) and Prebiotic Fiber Source (0.5 % oat flour) up to 21 Days of Storage. The pH, Acidity, Syneresis, Protein, Fat and Crude Fiber Content of the Synbiotic Yogurt was High among all the Other Yogurt Samples.	[64]

Products	Probiotic	Prebiotic	Use of Prebiotic and Their Effect in Synbiotic Products	References
Synbiotic Mousse	*Lb. acidophilus* La-5	Inulin and Fructo Oligosaccharides	Significant Reductions of Total Cholesterol and HDL-Cholesterol, as well as of Immunoglobulins (A and M), and Interleukin-1β in both Groups	[65]
Synbiotic Low-Fat Yogurt	*Lb. casei*	Pectinase Hydrolyzed Fraction of Tragacanth Gum (PHFTG)	Low Molecular Weight Tragacanth Gum (TG) was Easily Fermented as Indicated by Concomitant Increases in *L. casei* Population. TG can act as Substitutes for Conventional Stabilizers or Fat Replacer in the Food Industry that can also Enhance the Health Benefits of Products.	[66]

Genetic Improvement of Probiotics for Enhancing the Health Attributes: According to the Food and Drug Administration (FDA), Bifidobacteria and Lactic Acid Bacteria are Generally Recognized as Safe (GRAS). Currently, a global area of active research revolves around U.S. application of genetic engineering technology, specifically to develop new strains or improve previously characterized ones. Gene technology advances have allowed us to modify strains by introducing new genes or altering their metabolic functions. There have been numerous ways to introduce heterologous genes into Lactic Acid Bacteria strains that have been demonstrated over the last 20 years [67].

Through means of recombination, chromosomal integration, as well as the development of CRISPR-Cas9 genome editing in combination with ssDNA recombineering, Kojic *et al*. could express the aggregation factors of *L. lactis* in the probiotic bacteria with an interest, to enhance its adhesion to the intestinal epithelium [68]. Vaccines centered around probiotic bacteria could be manufactured and produced, and human cytokines in the strains could be increased as well to promote immune system stimulation [71]. Enzyme expression in probiotic bacteria, such as bile salt hydrolase from *Lactobacillus fermentum,* could help decrease cholesterol levels, and patients with celiac disease could reduce gastrointestinal problems through expressing a prolyl endopeptidase in probiotic bacteria [69]. The CRISPR system has only modified a few nucleotides in Lactic Bacteria [70]. However, through the development and improvement of this technique, we could allow for much longer gene sequences to be edited, allowing us to introduce new heterologous genes in Lactic Acid Bacteria and Bifidobacteria [71]. CRISPR modified organisms are not considered GMOs but are evaluated to be safe and acceptable in food manufacturing and human health.

In the 2011 article, *Integrative Expression System for Delivery of Antibody Fragments by Lactobacilli*, Martín *et al*. described an expression system that was chromosomally integrated in *Lactobacillus paracasei*, based on the *apf* gene of *Lactobacillus crispatus,* an aggregation-promoting factor gene [72]. *Lb. paracasei* was shown to produce antibodies directed against the rotavirus. Alvarez-Sieiro *et al*. engineered a food-grade strain of *Lb. casei* to deliver *Myxococcus Xanthus* Prolyl Endopeptidases into the gut environment [72]. Steidler *et al.* also showed that *L. lactis* strains could treat inflammation in mouse colitis models through the expression and secretion of murine interleukin-10 (IL-10) [73]. Dutch authorities approved the use of *L. lactis* strains secreting human IL-10 as an experimental therapy in a small clinical trial for use in humans with inflammatory bowel diseases [74]. In the 1996 article, "Effect of ilvBN-Encoded α-Acetolactate Synthase Expression on Diacetyl Production in Lactococcus Lactis," Benson *et al*. reported that the level of Diacetyl (0.53

mM) was achieved by a combination of aldB (Alpha-Acetolactate Decarboxylase) deletion and ilvBN (Alpha-Acetolactate Synthase form Part of the Operon Involved in the Biosynthesis of Isoleucine, Leucine, and Valine). Increased expression compares favorably with the level produced by *L. lactis* ssp. *Lactis* Biovar *Diacetylactis* (0.087 to 0.17 mM) from Citrate fermentation. At a pH of 6.8, expression of the *als* gene in *L. lactis* NZ2700 caused more than 60% conversion of Pyruvate into Acetoin when aerobically cultivated, however, Butanediol was not formed [68]. Additionally, acetoin was obtained in similar amounts at a pH of 6.0, but under these conditions, approximately 20% of the Pyruvate was now converted into Butanediol. It has been indicated through various metabolic engineering studies that 80% or more of Lactose can be converted by the action of overproduced a-Acetolactate Synthase in *L. lactis* [75].

CONCLUSION

Probiotics are becoming increasingly popular in the society. Solutions that maintain societal well-being and reduces the disease burden is most welcome by the society and the governments. The consumption of foods with healthy microbes is not new to the world, but preparing novel products with clinically tested probiotics is more specific and reliable to give desired health attribute. Probiotics can be consumed as live cells in the form of a pill, but their consumption through the food system brings about more benefits. Therefore, the incorporation of probiotics in different food matrices is important. Probiotic foods will deliver the inherent nutrients present in the carrier foods apart from metabolites produced by culture. Probiotics in fermented milks are the best carrier through food matrix and have contribute various health benefits since its discovery. They also help in improving the biological and functional values of the food. There is a need to explore more customized probiotic milk products for managing the lifestyle diseases.

List of abbreviations: WHO: World Health Organization, FAO: Food Agricultural Organization, ISAPP: International Scientific Association for Probiotics and Prebiotics, DVS: Direct Vat Set, CAGR**:** Compound Annual Growth Rate, ILSI: International Life Sciences Institute, TAG: Triacylglycerol, LPP: Lipid Profile Parameters, NDRI: National Dairy Research Institute, WPC: Whey Proteins Concentrate, ACE: Angiotensin-Converting Enzyme, FOS: Fructo-Oligosaccharides, PHFTG: Pectinase Hydrolysed Fraction of Tragacanth Gum, FDA: Food and Drug Administration, GRAS: Generally Recognized as Safe, CRISPR: Clustered regularly interspaced short palindromic repeats, IL-10: Interleukin-10, LDH: Lactate dehydrogenase.

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