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Role and Current Trends of Developing Fruit, Vegetable and Cereal based Probiotic Foods: A review

Omanthamudalige Don Antoinette Niranjalie Perera[1*], Hewa Lunuwilage Darshani Weerahewa[2] and Sashikala Vidanagamage[2]

[1]Department of Food Science and Technology, Faculty of Livestock Fisheries and Nutrition, Wayamba University of Sri Lanka, Sri Lanka
[2]Department of Botany, Faculty of Natural Science, The Open University of Sri Lanka, Sri Lanka

*Corresponding author: aannpp2003@gmail.com

ABSTRACT

Scientific investigators have motivated recently from principal role of food by supplying adequate nutrients and energy to sustain physiological functions and well-being. Conversely, the consumer interest about the active role of food in well-being and life prolongation has been improved. In this manner, a novel term functional food was introduced which refers to the prevention and therapeutic effects of food beyond its nutritional value. A wide array of functional foods has been developed recently and many of them are being produced in all over the world i.e. probiotic, prebiotic and synbiotic foods. All these functional components are able to exercise substantial influences on human well-being. The present review focuses on recent developments in dairy and non-dairy probiotic products. All over the world, dairy probiotics are being commercialized in several different forms. However, the allergy and lactose intolerance are the major health concerns to dairy probiotics. Whereas, flavor and refreshing nature are the principle advantages of non-dairy drinks, especially fruit juices. Emphasizing these positive activities is one possible approach for improving the health image of non-dairy fermented products and developing the functional foods. Discovering of new probiotic/prebiotic/synbiotic functional foods is linked to the interest of the food industry to renovate constantly through introduction of products with enhanced nutritional value, but also with health advantage for consumers.

Keywords: Functional food, Dairy and non-dairy products, Probiotics, Prebiotics, Synbiotics

A food can be considered as a functional nutrition when it shows beneficial effects on one or more target functions in the body (Reis *et al*. 2017). During the last span, knowledge of the significance of diet in human health and well-being has considerably increased (Panghal *et al.* 2017) and nutritionists have identified specific foods playing a key-role in supporting the consumers' health status. Beyond meeting nutritional requirements, it is extensively recognized that dietary factors are able to change the detrimental development of different chronic diseases (Boludaand Vidal-capilla, 2017; Alkewi, 2014; Peiretti *et al.* 2015). The increasing awareness on healthy diets and demand for health and wellness food products opens a great opportunity for producers of Sri Lanka and worldwide. With a heritage of natural and ayurveda products, Sri Lanka should be well placed to provide to this growing consumer segment.

Functional food science will assist to establish claims based on either increasing its function or decreasing risk of diseases (Yamamoto, 2017; Grajek *et al.* 2005). The most well-known food-based strategies to modulate the composition of the intestinal microbiota are the dietary practices of prebiotics, probiotics and their mixtures as synbiotics (Bernal *et al*. 2017; Roberfroid, 2002). Currently established prebiotic compounds are primarily targeting the bifidobacteria population of the colon microbiota. A virtuous illustration of the importance of high colonic bifidobacteria levels is that breast milk, creates an environment in the colon favouring the development of a simple flora, dominated by bifidobacteria to which various health benefits have been endorsed (Deng *et al*. 2015).

Bacterial cultures, called as starters, are used in the manufacture of yoghurt, kefir and other cultured milk products. The starters are added to the product and allowed to grow under controlled conditions. In the sequence of fermentation, the bacteria produce fermented product having characteristic properties such as acidity (pH), flavour, aroma and consistency. The drop in pH, due to the bacterial fermentation of lactose to lactic acid, has a preservative effect on the product and nutritional value and digestibility are improved simultaneously. But, manufacturing the pure starter culture is one of the most vital and difficult process in dairy industry.

For centuries, the preservation and storage of fermented foods involving cereals, soya, meat, etc., are being experienced. Though, fermentation process involves mixed cultures such as yeasts, lactic acid bacteria (LAB) and fungi (Misihairabgwi and Cheikhyossef, 2017; Blandino, 2003) and traditional fermented foods are the potential source of microorganisms and show probiotic characteristics. Still, some health risks are associated with milk based probiotic foods (Saarela, 2002). To lessen these risks of dairy fermented foods, several non-dairy fermented foods have been developed worldwide (Shori, 2016; Rivera-Espinoza and Gallardo-Navarro, 2010). Among the non-dairy based fermented foods, fruits and vegetables, cereals and soy based foods are being prominent (Martins, 2013).

Thus, the development of synbiotics; combination of probiotic and prebiotic is a best technical approach of functional food industry. This review delivers an overview of probiotics, prebiotics and synbiotics, increasingly popular ingredients that can be found in functional foods and dietary supplements.

Probiotics

Probiotics are "live microorganisms, when administered in adequate amounts; confer a health benefit on the host" (FAO/WHO, 2002). Over the years, many other definitions have been suggested, but this definition is most generally accepted in the scientific community. Most popular microorganisms are lactic acid bacteria for example *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus acidophilus, and Streptococcus lactis* (Sindhu and Khetarpaul, 2001). But other organisms containing enterococci and yeasts have also been used as probiotics. Bifidobacteria are a major group of probiotic microorganisms, which have been widely researched for their probiotic properties (Prasanna and Charalamppoulos, 2018).

Probiotic bacteria have been used to treat or prevent a wide range of human diseases and syndromes (Parker *et al*. 2017). Research has shown that addition of probiotics to food provides some health benefits such as reduction of lactose intolerance, reduction of cholesterol levels, immune system stimulation, relief from constipation, increased absorption of minerals, antimutagenic, anticarcinogenic, and antihypertensive effects (Pereira and Rodrigues, 2018) and it has been suggested that probiotic bacteria can improve digestive function, reduce diarrhea associated with antibiotic therapy, helps in reducing the risk of certain acute common infectious diseases, improve tolerance to lactose and enhance immune functions, reduce crying time in colicky babies (Panghal *et al*. 2017; Rafter *et al*. 2007; Saarela, 2002; McNaught and MacFie, 2001; Berner and Donnell, 1998).

The clinical uses of probiotics are wide-ranging; (Table 1) however, the clinical indications based on evidence-based studies are much narrower and are open to continuing evaluation. Scientists are studying day by day about the role of microbes on people's health and the respective type with the precise level of probiotic microbes.

Table 1: List of human diseases and conditions that probiotics have been used to prevent and/or treat

Medical Condition	Class(es) of probiotic
Lactose mal digestion	Lactic acid bacteria (LAB) and *Streptococcus salivarius* sub sp. *thermophilus*
Gastroenteritis Acute diarrhea	LAB, *Bifidobacterium* species, or *Saccharomyces boulardii*
Antibiotic-associated diarrhea	LAB or *S. boulardii*
Traveler's diarrhea	LAB
Allergies	LAB
Clostridium difficile-induced colitis	LAB
Dental caries	LAB
Intestinal inflammation in children with cystic fibrosis	LAB
Respiratory infection in children	LAB
Nasal colonization with pathogens	LAB
Inflammatory bowel disease or irritable bowel syndrome	LAB and *Bifidobacterium* species, *S. boulardii* and drug, *S. boulardii* alone, or LAB alone

Special focus on non-dairy probiotic products

Most of the probiotic foods originate on dairy basis which consists high in fat content, but consumers' preference presently lies more with healthy dietary supplements. Mostly, health benefits of probiotics are fulfilled by milk and other dairy products, however lactose intolerance, cholesterol content and allergic milk proteins are limiting factors in growth of dairy probiotics (Panghal *et al*. 2017). Research is being continued in developing alternate solutions to dairy based probiotic products and preference for non-dairy based probiotic products particularly using fruit and/or vegetable juice as a major ingredient (Tuorila and Cardello, 2002). The above fact is highlighted by tendency in the U.S. functional food market, which is developing in a different fashion from that seen in Europe, with its functional food sector more broadly defined as nutraceuticals and consumer interest tending to lie more with botanical dietary supplements rather than fortification of foodstuffs (Luckow and Delahunty, 2004).

Botanical supplements offer many advantages, they are rich source of nutrients and unlike in dairy products, it obviates the necessity of using starter cultures and hence no competition for nutrients with probiotic cultures. Non-dairy sources are fortified with acidulants which could enhance the shelf-life by creating an anaerobic environment which is more optimal for probiotic cultures that is attained by scavenging the available oxygen. Fruit juices contain sugars also to support the growth of probiotics (Ding and Shah 2008). Fruit juices have been shown to be suitable substrates for probiotic bacteria cultivation, as they already contain beneficial nutrients, such as minerals, vitamins, fiber, and antioxidants. Also, fruit juices have the advantage of having a pleasant taste appreciated by all age groups, and they are perceived as a healthy and different food (Pereira & Rodrigues, 2018). Another benefit is that these juices stay very less time in the stomach and thus the probiotic species spend very less time to the harsh acidic environment of the stomach.

Besides dietary practices, major health concerns related to the fermented dairy products; lactose intolerance and the high cholesterol content (Heenan *et al.* 2004) discourage many Asian people from consuming milk. The average per capita dairy consumption in the last 10 years for the major Asian dairy markets was ranged about 10 kg to 100 kg (Dong, 2006) and it was considerably high in European countries with 300 kg. Therefore, considering the above-mentioned evidences cereals, fruits and vegetable may be potential substrates, where the healthy probiotic bacteria will play a role in both in developing and developed countries.

Wide range of traditional non-dairy fermented beverages produced around the world. Much of them are non-alcoholic beverages produced with cereals as the principle raw material. The non-dairy probiotics beverages may be made from a variety of raw materials, such as cereals, millets, legumes, fruits and vegetables (Table 2). Studies may be categorized based on the source of raw material for the production of the non-dairy probiotic beverage.

Table 2: List of some commonly available dairy/non-dairy probiotic products developed recently

Category	Product
Dairy based	Acidophilus milk
	Acido-whey, Ice-cream
	Lassi
	Cheese
	Curd
	Non fermented goat's milk beverage
	Frozen synbiotic dessert
	Yoghurt
Fruit and vegetable based	Fermented banana Beet-based drink Tomato-based drink Peanut milk Ginger juice
	Cabbage, carrot juices
	Probiotic banana puree
	Fruit juices (mango, grape, cranberry, pineapple and orange)
Soy based	Non-fermented soy-based frozen desserts and fermented soymilk drink
	Soy-based stirred yogurt-like drinks
	Soy based products
	Soyghurt
	Soy curd
	Soy product fermented with Kefir
Cereal based	Cereal-based puddings
	Rice-based yoghurt
	Oat milk
	Oat, barley and malt based
	Wheat, rye, millet, maize and other cereals fermented probiotic beverages
	Mixed cereal beverage
	Bread and baked products
	Sorghum based 'Sorghurt'

Prebiotics

Prebiotic is "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health (Bernal *et al.* 2017; Gibson and Roberfroid, 1995). A group of prebiotics with various origin and chemical properties reviewed the prevailing prebiotics and classified them based on a set of common criteria (Stowell, 2007); (a) Resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption (b) Fermentation by intestinal microflora (c) Selective stimulation of the growth and/or activity of intestinal bacteria.

Prebiotics that fulfill these three criteria are as follows, Inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactulose and polydextrose are recognized as the conventional prebiotics, whereas isomaltooligosaccharides (IMO), xylooligosaccahrides (XOS), and lactitolare characterized as the emerging prebiotics. Mannitol, maltodextrin, raffinose, lactulose, and sorbitol are also prebiotics with proven health properties (Yeo and Liong 2010; Vamanu and Vamanu, 2010; Mandal *et al.* 2010). Resistant starch-rich whole grains can be considered as prebiotic in nature and expected their consumption leads to various health benefits. These are not absorbed in small intestine of healthy individuals but later are fermented by natural microflora of the colon to produce short-chain fatty acids (Vaidya and Sheth, 2010).

Prebiotics are found in several vegetables and fruits and are considered to be functional food components with vital technological advantages. Their incorporation develops sensory characteristics such as taste and texture, and enriches the stability of foams, emulsions and mouth feeling a large array of food applications like dairy products.

Synbiotics

Synbiotics refers to nutritional supplements combining Probiotics and Prebiotics (Yu *et al*. 2016) that are supposed to perform together in a form of synergism, hence synbiotics, enhancing their isolated beneficial effects (Roberfroid, 2002). When two nutritional ingredients or supplements are given together give positive results of one of three patterns; additive effect, synergism or potentiation. Additive effect occurs when the effect of two ingredients used together approaches to the sum of the individual ingredient effects. It has been suggested that the combination of a probiotic and a prebiotic, might be more active than either a probiotic or prebiotic administered alone (Asemi *et al.* 2015; Roberfroid, 2002).

Though the benefits of synbiotic therapy are noticeable, it is difficult to determine the best combination for each disease and each individual, as it is link with probiotics and prebiotics alone. Synbiotics affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria (Geier *et al*. 2007: Tinello *et al.* 2017)

In terms of nutritional benefits associated with microflora, foods are the main vehicle for probiotic, prebiotic and synbiotics. So, development of synbiotics is a valuable area of enhanced functional food compounds. Scientists are extremely interested in symbiotic concept as it leads to the combination of probiotics and prebiotics. The influence is directed towards two different target traits of the gut, both the small and large intestinal tracts as probiotics are mainly active in the human small intestine whereas prebiotics are only effective in the large intestine, so the combination of the two may give a synergistic effect (Huynh *et al*. 2017; Roberfroid 2002; Gibson and Roberfroid, 1995). Prebiotic oligosaccharides stimulate probiotic bacteria in the colon, also prebiotic carbohydrate is used by a probiotic strain for its growth and replication in the gut will be selectively supported (Deng *et al.* 2015). This mixture could enhance the survival of probiotic bacteria, due to the healthier host composition and availability of specific substrate for fermentation.

There are many evidences related to animal studies on the potential positive effects of synbiotics; in one of the comparative in vitro studies a number of strains of *Bifidobacterium longum, Bifidobacterium animalis* and *Bifidobacterium catenulatum* were found grew best on FOS with much more lower growth rate reports on inulin. Specific synbiotics were provided to rat or chicken and their faeces were characterized for coliforms, bifidobacteria, and total cell counts (Mousavi *et al*. 2015; Paturi *et al.* 2015). Greater levels of coliforms and bifidobacteria were found in animals fed both FOS and synbiotics (Bielecka *et al*. 2002). Synbiotics are believed to intensify the persistence of probiotics in gut, was supported by studies including the preparation of *Lactobacillus acidophilus* and FOS has been investigated as in vitro model of human gut (Gmeiner *et al.* 2000). Moreover, it has been assumed that synbiotics consumption decrease health risk factorsin patients with colon cancer (Rafter, 2003). Animal studies reported that combining probiotic and prebiotic give protective effect against the development of tumor in colon. But, there were few of human evidences supporting this recommendation (Liong, 2008).

Finally, it is vital to select a mixture of a definite substrate and a microorganism for a synbiotic product that can enhance the advantageous effect when compared to a product including a probiotic or a prebiotic only (Capela *et al.* 2006; Huebner *et al.* 2007).

DISCUSSION

Fermented dairy products have been the food vehicles with the biggest technological and commercial attainment for the incorporation of probiotic bacteria. Despite this success of dairy probiotics, consumers have a genuine interest in non-dairy based functional beverages prepared with probiotics because they offer varied taste profiles that are appealing to all age groups and also they are perceived as healthy and refreshing in contrast to dairy foods. The requirement of non-dairy probiotic products and their production are not going together compared to dairy probiotics. The current research is a rising at an attractively but not satisfactorily enough when compared to their dairy equivalents. Development of novel, economic and technological ideas is an appropriate solution to bring the non-dairy probiotic foods on similar level of dairy probiotic foods.

Over the past two decades many food producers around the world, especially dairies have successfully launched products containing well-documented probiotic strains. Among them Chris-Hansen have introduced a novel bacterial strain *L. casei431*® and the research carried out by them indicated a probiotic bacteria *L. casei431*® survive in the low acid juices up to at least 8 weeks. *L casei431*® incorporated juice has been designed to give the best results in many types of healthy fruit juices from citrus, apples, pears, berries and coconuts (www.chr-hansen.com). Although there is an excessive potential for the use of fruit juices as probiotic products, very few research evidences on their preparation and production are available.

Based on the research carried out by *L.casei 431*®it can be found that carrot juice is a good substrate for the growth of *L. casei431*®, and can be utilized in the production of fermented good quality synbiotic beverage with acceptable sensory characteristics up to six weeks of storage under refrigerated (5 ± 1°C) conditions (Alwis *et al*. 2016). In addition, research has proven the better survivability of probiotic *L.casei431*®in finger millet based beverage. Results have revealed that sensory acceptable finger millet based fermented probiotic beverage can be developed using *L.casei 431*® by inoculating 0.031 g L^{-1} inoculum of frozen *L.casei 431*® and could serve as a ready to drink functional beverage at refrigerated (5 ± 1 °C) storage up to 5 weeks (Fasreen *et al.* 2017). The count of probiotic organism for both beverages was within the level of standards (10^8CFU/ mL) until the end of storage period. Another study revealed that beetroot could serve as a raw material for the production of probiotic beet juice through lactic acid fermentation with *L.casei 431*® (Gamage *et al*. 2016). The fermented beetroot juice has a pH value < 5.5 and contains a significant number of beneficial lactic acid bacteria (10^8CFU/mL). Synbiotic beetroot beverage also has shown acceptable sensory characteristics to healthy consumers.

Furthermore, the recent study was conducted to assess the effect of lactic acid fermentation on the phytochemical, volatile and sensory attributes of mulberry juice (Emmanuel *et al*. 2017). The results showed that LAB were able to improve the phytochemical compositions, volatile profile of the juice and sensory acceptability of the mulberry juice. With reference to the performance among three LAB strains; *L. plantarum, L. acidophilus* and *L. paracasei,* the strain of *L. plantarum* was observed to be the most suitable for fermentation of mulberry juice. Moreover, anew fermented almond milk was developed using *L.reuteri and Streptococcus thermophilus* and different factors (glucose, fructose, inulin and starters) were optimized to assure high probiotic survivals in the final product (Bernet, 2015). Results showed that a high probiotic population (>10^7cfu/mL) was obtained in the previously optimized almond milk throughout cold storage, which correspond to the adulterants and probiotic bacteria significantly survived (51%) in vitro digestion, related to the presence of inulin, which would enhance the potential health benefits of its consumption.

Future perspectives

Firstly, the efficacy of the bacteria involved in non-dairy drinks must be tested in vitro and vivo conditions, since lack of much positive evidence suggests the necessity of pure results to confirm its worth. Apart from the functional properties, sensory acceptance especially; taste, appeal and price are the special characteristics when they are available in commercially. Selection of cultures with potential probiotic properties plays a key role in the success of the non-dairy probiotic products since, all cultures or strains may not have probiotic properties. The activity of probiotics in food applications depends on factors like water activity, processing and storage temperature, shelf life, oxygen content, pH, mechanical stress, salt content, and content of other harmful or essential ingredients (Rodgers, 2001). Even though, fruit juices have been shown as an appropriate carrier for probiotics, the limiting factor for many probiotic strains is maintaining appropriate pH of the juices.

It is needed that above technological issues that can affect the survival of probiotic cultures throughout the production process and during storage should also be addressed while formulating new probiotic products. Therefore, precautions should be taken while confirming the functional features of starters before incorporating in to the product.

CONCLUSION

There is a wide variety of functional foods that were developed recently and many of them are being produced in globally; probiotic, prebiotic and synbiotic foods. Most of the probiotics products are milk based. However, the increase in consumer vegetarianism and demand for cholesterol free probiotics has stimulated researchers to explore new medium for probiotics, among them fruit and vegetable based, cereals based and soy based foods are prominent. These foods contain beneficial nutrients, such as minerals, vitamins, dietary fibers and antioxidants and also lack of allergens which making them ideal substrates for probiotic cultures. Even supposing there is growing interest in consumers towards healthier foods and probiotic fruit, vegetable juices and cereal based beverages can serve as a good option. But, the application of probiotic cultures in non-dairy products and their environmental safety are great challenge and research should be done extensively before commercialization.

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