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REVIEW PAPER

Synbiotics: A culinary Art to Creative Health Foods

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Abstract

Fermentation has always remained the most reliable technique to develop new exotic health foods. The probiotic drinks has been in the market for over a decade and are well known for their health promoting functionalities. Recently, the importance of prebiotic factors like xylobiose, inulin, lactulose etc. in promoting the growth of probiotic microorganisms inside the colon has been emphasized and has become an emerging area of research. The new approach in this area is the development of synbiotic drinks which are known to impart the beneficial effects of both probiotics and prebiotics. Synbiotics have more unique and promising ability to impart health benefits over the probiotics. Many traditional foods like *kanji, lugri, pozol, sufu, bushera* etc. are known to possess synbiotic behavior. There is a tremendous scope to develop new exotic synbiotic foods by combining the traditional knowledge and modern science. The relevant issues with respect to the synbiotics foods have been reviewed here.

Keywords: Synbiotic, probiotic, prebiotic, indigenous fermented food, health foods

Fermented foods are always healthier than their unfermented counterparts (Joshi, *et al.* 1999). The latest in the series of fermented foods are the synbiotics. The term 'synbiotic' is defined as a mixture of prebiotic and probiotic (Rizal *et al.,* 2011). Synbiotic foods contain probiotic as bacteria of health significance, a source of prebiotic and sugars as source of nutrition for the growth of bacteria that beneficially affect the host by improving the survival and implantation of live microbial dietary supplement in gastrointestinal tract (Gibson and Roberfoid, 1995).

Although the term "synbiotic" is new but such foods have been produced since antiquity without knowing the actual science in valved. Almost every region or culture has their own traditional synbiotic drinks/foods depending on its locality and availability of raw material. A variety of traditional foods such as *toddy, lugri, bushera, pozol* etc. are known to contain live probiotic microorganisms like *Lactobacillus* spp thereby exhibiting synbiotic properties. Modern research has emphasized the importance of synbiotics in human health. In April 2013, Beef Cattle Research Council funded a study at USD 500,000 for exploring the use of synbiotics in the cattle industry which is expected to force manufacturers to increase R&D expenditure on new product developments. For improving gut and digestitive health Sabinsa and Fruit D'or Nutraceuticals formed a strategic partnership to develop a new synbiotics product 'Lactocran' in December 2014. Major players in the symbiotic/synbiotic market are Daflorn, Chr. Hansen, Skystone Feed, Newleaf, Behn Meyer, Yakult Pharmaceuticals and Pfizer (Anonymous, 2015).

Growing demand for dietary supplements in China and India on account of increasing awareness about nutritional benefits of functional foods is expected to have a positive impact on synbiotics market over 2015-2022. Asia Pacific is expected to be one of the promising markets for synbiotics on account of dairy industry growth in China and India (Anonymous, 2015).

Rationale for the use of synbiotics: Functional-Structural relationship

It is an established fact that the human gastrointestinal (GI) tract behaves like a kinetic micro-ecosystem that maintains normal physiological functions of the host organism (Table 1a and b). Systematic supplementation of the diet with probiotics, prebiotics or synbiotics ensures a proper equilibrium of the gutmicroflora (Bielecka *et al.,* 2002).

Table 1 (a): The common inhabitants of human gut

Bacteria	*Bacteroides* (30%), *Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Bifidobacterium*
Yeasts	*Candida, Saccharomyces, Aspergillus, Penicillum*

Table 1 (b) Concentration of microflora in different parts of human gut

Locale of microflora	*Population*	*Species*	*References*
Small Intestine	10^3 cfu/g	Aerobic microbes	Fooks *et al.*, 1999
Large bowel	10^{10} to 10^{11} cfu/g	*Bacteroides, Bifidobacterium, Eubacterium, Streptococcus, Enterococcus, Lactobacillus, Peptococcus, Peptosyrptococcus, Ruminococcus, Acetogens, Candida, Saccharomyces, Aspergillus, Penicillum*	Topping and Clifton 2001; Fooks *et al.*, 1999

Probiotics

Probiotics are defined as the "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2006). These microorganisms have been (Table 2) used to improve the health of both animals and humans through the modulation of the intestinal microbiota. At present, in order to reduce the risk of gastrointestinal infections or treat such infections, several well-characterized strains of *Lactobacilli* and *Bifidobacteria* are available for human use (Salminen *et al.,* 2005). Resistance to enteric pathogens is also increased by probiotics through antagonistic action, adjuvant effect, increasing antibody production and resistance to colony formation on body surfaces, such as GI, the urogenital and the respiratory tract (Nagpal *et al.,* 2012).

Certain species of bacteria produce different organic acids (Table 3) along with other substances which have positive impact on health. *Bifidobacteria* produce acetic and lactic acids in a molar ratio of 3: 2. *Lactobacillus acidophilus* and *L.casei* produce lactic acid as the main end product of fermentation. In addition to lactic and acetic acids other acids such as hippuric and citric acid are also known to be produced. Lactic acid bacteria also produce hydrogen peroxide, diacetyl, and bacteriocin as antimicrobial substances (Joshi and Sharma 2012). These inhibitory substances create antagonistic environments for foodborne pathogens and spoilage organisms (Dave and Shah, 1997).

Addition of *L. acidophilus, Bifidobacterium longum, Lactobacillus GG, L. acidophilus, Lactobacillus gasseri, Lactobacillus confusus, Streptococcus thermophilus, Bifidobacterium breve* and *B. longum* in the diet were found to have anti-cancerous properties. The possible mechanisms of action include inhibition of intestinal bacterial enzymes that convert pro-carcinogens to more proximal carcinogens (Kumar *et al.,* 2011a, b), reducing the expression level of *ras-p21* expression and cell proliferation, affecting the expression of genes involved in immune response and inflammation, apoptosis, cell growth and cell differentiation (cyclins and caspases, oncogenes), cell–cell signaling (intracellular adhesion molecules and integrins), cell adhesion (cadherins), signal transcription and transduction (Caro *et al.,* 2005).

Lactic acid bacteria may inhibit colon cancer by enhancing the host's immune response, altering the metabolic activity of the intestinal microbiota, binding and degrading carcinogens, producing antimutagenic compounds and altering the physio-chemical conditions in the colon (Hirayama and Rafter, 2000; Kumar *et al.,* 2011a, b).

Pre-biotics

Probiotics also have cholesterol lowering effects. St-Onge *et al.,* (2000) extensively reviewed the existing studies which concluded that the consumption of fermented products containing probiotic bacteria contributed to cholesterol lowering effect. Similar studies by Gopal *et al.* (2001) have also reported cholesterol reduction by *Bifidobacterium* spp and *L. acidophilus*. The possible mechanisms of action for cholesterol reduction include cholesterol assimilation by bacteria, conjugation of bile salts, cholesterol binding to bacterial cell walls, and reduction in cholesterol biosynthesis (Pereira and Gibson, 2002).

Some of the foods marketed as probiotics foods in India are listed in Table 4.

Table 2: Genera and species of microbes studied and used as probiotics

Genus	Species
Lactobacillus	*acidophilus* *brevis* *delbrueckii* *fermentum* *gasseri* *johnsonii* *paracasei* *plantarum* *reuteri* *rhamnosus* *salivarius*
Bifidobacterium	
	adolescentis *animalis* *bifidum* *breve* *infantis* *longum*
Streptococcus	*thermophilus* *salivarius*
Enterococcus	*faecium*
Escherichia	*coli*
Bacillus	*coagulans* *clausii*
Leuconostoc	
Pediococcus	
Propionibacterium	
yeasts and moulds	
Saccharomyces	*cerevisiae* boulardii
Aspergillus	*cerevisiae* *niger* *oryzae*

Source: Modified from Anuradha and Rajeshwari, 2005.

Table 3: Organic compounds produced by colonic microflora

Microorganism	Organic compound	References
Bifidobacteria, Bifidobacterium b-galactosidase	*Lactic acids* Transgalacto-oligosaccharides	Fooks *et al.*, 1999
Lactobacillus acidophilus, Lactobacillus casei Lactobacillus sanfranciscensis	*Lactic acid (main product), Acetic acid, hippuric acid, citric acid, Hydrogen peroxide, diacetyl, bacteriocin,* Extracellular laevans	Dave and Shah, 1997

Table 4: Commercial Probiotic foods available in India

Probiotic Foods	Company
Probiotic curd	Heritage Foods (India) Ltd.
'b-Activ' probiotic curd (L. acidophilus and B. lactis strain BB12)	Mother Dairy
'Nesvita' Probiotic yoghurt	Nestle
Probiotic ice creams, 'Amul Prolife', 'Prolite' and 'Amul Sugar free'	Amul
Yakult, Probiotic curd with *L.casei* strain *Shirota*	Yakult Danone India (YDI)
Fructo-Oligo Saccharides, Probiotic drugs	Glenmark Alkem Labs

The term 'prebiotic' was introduced by Gibson and Roberfroid (1995) who exchanged "pro" for "pre," which means "before" or "for". Whole cereal grains, fruits and vegetables being rich sources of fibre, vitamins, and minerals provide the essential prebiotic factors for the growth of probiotic microorganisms. Along with these prebiotic factors they also contain bioactive phytochemicals, that provide desirable health benefits beyond the basic nutrition due to their additive and synergistic effects. Also, many ornamental flowers are natural sources of very important bioactive compounds with benefit to the human health and their possible role as dietary components has also been reported (Cavaiuolo *et al.,* 2013).

The prebiotic factors are generally non-digestible or low-digestible food ingredients that selectively stimulate the growth and activity of one or a limited number of probiotic microorganisms in the colon (Manning and Gibbson, 2004). Prebiotics include monosachharides, fructo-oligosaccharides, galacto-oligosachharides etc. (Table 5). The soybean oligosaccharides raffinose and stachyose are well known prebiotics. Soygerm powder has been investigated as a source of these prebiotics and is also rich in isoflavones (De Boever *et al.,* 2000). Arabinogalacto-oligosaccharides can be made from soybeans by endogalactanases, arabino-oligosaccharides can be made from sugar beet by endoarabinanases, rhamnogalacturono-oligosaccharides can be made from apple by rhamnogalacturonases, arabinoxylo-oligosaccharides can be made from wheat by xylanases and galacturono-oligosaccharides can be made from polygalacturonic acid by endogalacturonases (Rastall and Maitin, 2002).

Table 5: Prebiotic Oligosachharides

Fructo-oligosaccharides
→ Inulin
Galacto-oligosaccharides
→ Lactulose
→ Lactosucrose
Xylo-oligosaccharides
→ xylobiose
Isomalto-oligosaccharides
Soybean oligosaccharides
→ Arabinogalacto-oligosaccharides
Gentio-oligosaccharides
Transgalactosylated oligosaccharides

Source: Modified from Rastall and Maitin, 2002.

Synbiotic

The term synbiotic alludes to synergism in which the prebiotic compound selectively favours the growth of probiotic factors (Figure 1). Several compounds have been reported in favour to support the growth of probiotic microorganisms. Fractionated galacto-oligosachharides were used as growth substrates for *Bacillus lactis* and for *L. rhamnosus* (Gopal*et al.,* 2001). *L. rhamnosus* preferred monosaccharides and disaccharides as substrate, whereas *B. lactis* grew preferentially on the trisaccharide and tetrasaccharide fractions, even preferring these oligosaccharides to glucose. It seems likely that *B. lactis* has a specific transport system for galacto-oligosachharides that is not present in *L. rhamnosus*. A galactosidase enzyme that displays hydrolytic activity towards galacto-oligosachharides, but not lactose, has been described in *Bifidobacterium adolescentis* (Van Laere, 2000). Prebiotic-probiotic synergism, has been ilustrated by inulin was found to support larger total bacterial counts but lower populations of bifidobacteria and lactobacilli than soybean oligosaccharides, isomalto-oligosaccharides or lactulose. Fructo-oligosachharides favoured higher populations of *streptococci* than did galacto-oligosachharides or soybean oligosaccharides.

The largest increase in *Bifidobacterium* was seen on xylo-oligosaccharides and lactulose, the largest increase in *Lactobacillus* was on fructo-oligosaccharides and the largest decrease in clostridia was on galacto-oligosachharides. Fructo-oligosachharides has been found to be effective in humans at doses of 5 g/day, which resulted in a 1 log increase in *Bifidobacterium* relative to a sucrose *placebo* (Rycroft *et al.,* 2001). Ability of the prebiotics to protect probiotics from bile acid stress was also studied. Both *L. acidophilus* NCFM and *L. reuteri* NCIMB 11951 were able to grow in 2 mM cholic and taurocholic acid when incubated in synbiotic combinations with lactulose (1%) or lactobionic acid (1%) (Adebola *et al.,* 2014).

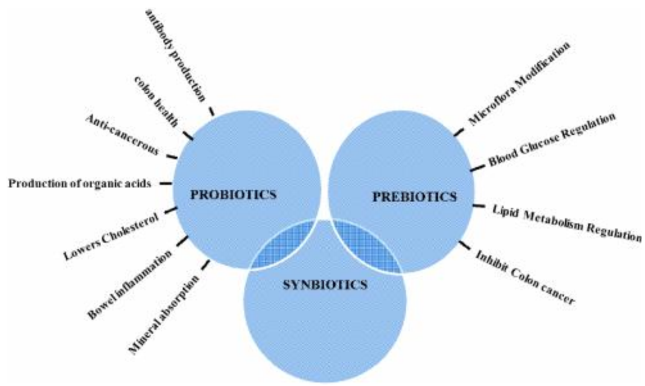

Fig. 1: Functions of the Synbiotic foods

Indigenous foods with synbiotic characteristics

Many researchers have worked on the indigenous fermented foods and have suggested the synbiotic characteristics of these foods and beverages as these are reported to have live beneficial microflora along with the prebiotic factors. Some of the major foods are reviewed here.

Raabadi, a cereal based fermented milk product is popular in rural Haryana, Rajasthan and Punjab. The cereals that are used for *raabadi* preparation are barley, wheat, maize, pearl millet, barley and/or sorghum. Traditionally, *raabadi* is prepared by adding cooked and cooled mixture of cereal flour to buttermilk and allowing the mixture to ferment at about 40-45^{o}C for 4-6 hrs. The product is consumed as such. It is lactic acid fermented food in which lactose undergoes acid fermentation naturally (Gupta 1989; Ramakrishnan 1977).

Tarhana is another traditional cereal based fermented food made in Turkey. It is prepared by mixing yoghurt, wheat flour, yeast and a variety of vegetables and spices followed by fermentation for 1-7 days. After fermentation, the mixture is sundried and ground. It has an acidic and sour taste with yeasty flavor, and is used for soup making (Ibanoglu and Ibanoglu, 1997).

Boza, a beverage consumed in Bulgaria, Albania, Turkey and Romania, is made from wheat, rye, millet, maize and other cereals mixed with sugar. It is a colloidal suspension from light to dark, sweet, slightly sharp to slightly sour. Microflora-identification of Bulgarian *boza* showed that these were mainly consist of yeasts and lactic acid bacteria, on an average LAB/yeast ratio of 2.4. The lactic acid bacteria isolated were *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus coprophilus*, *Leuconostoc reffinolactis*, *Leuconostoc mesenteroides* and *Lactobacillus brevis*. The yeasts isolated were *Saccharomyces cerevisiae*, *Candida tropicalis*, *Candida glabrata*, *Geotrichum penicillatum* and *Geotrichum candidum* (Gotcheva *et al.,* 2000 and Blandino *et al.,* 2003).

Bushera is a traditional beverage prepared in the Western highlands of Uganda, consumed both by the young children and the adults. The flours from germinated sorghum and millet grains is mixed with the boiling water and left to cool to ambient temperature. Germinated millet or sorghum flour is then added and the mixture is left to ferment at ambient temperature for 1–6 days. The lactic acid bacteria isolated from *bushera* comprised of five genera, *Lactobacillus*, *Lactococcus, Leuconostoc*, *Enterococcus* and *Streptococcus. Lactobacillus brevis* was more frequently isolated than other species (Muianja *et al.,* 2003).

Mahewu (*amahewu*) is a sour beverage made from the maize porridge, which is mixed with the water. The sorghum, millet malt, or wheat flour is then, added and left to ferment. It is consumed in Africa and some Arabian Gulf countries. The spontaneous fermentation process is carried out by the natural flora of the malt at the ambient temperature. The predominant microorganism found in African *mahewu* is *Lactococcuslactis* subsp. *Lactis* (Blandino, 2003).

A refreshing beverage, called *Pozol* consumed in the Southeastern Mexico, is made by cooking maize in an approximately 1% (w/v) lime solution, washing with water, grinding to make a dough known as nixtamal, shaping into balls, wrapping in banana leaves and leaving to ferment at ambient temperature for 12 hours 4 days. The fermented dough is suspended in the water and drunk. Some fibrous components are not completely solubilized by nixtamalization and thus, a sediment is present in the beverage when the dough is suspended in the water (Wacher *et al.,* 2000).

Lugri is a mild alcoholic beverage, prepared and consumed in many parts of Himachal Pradesh, India. It is made by cooking local varieties of rice, cooling them, mixing with *phab* (local inoculum), filled in earthen pots, and left for fermentation for 10-12 days. The fermented product is filtered and filled in bottles. The product has a low storage life of 2-3 days and is generally consumed fresh. The microbiological evaluation of the *lugri* confirmed the presence of *S. cerevisiae, Leuconostoc sp.* and *Lactobacillus sp.* (Thakur *et al.,* 2004).

Kanji is a lactic acid fermented carrot based drink consumed in the Northern India. It is prepared mainly from the locally produced deep purple/black carrots. *Kanji* production typically relies on spontaneous natural fermentation. *Kanji* is very popular and is considered to have cooling, smoothing properties and have high nutritional value. *Kanji* is a functional probiotic beverage because it retains all the nutrients and viable microbiota till the time of consumption. It is effective in the treatment of diarrhoea, lactose indigestion, constipation, colonic disorders and food allergy. The natural probiotic bacteria species found were: *Lactobacillus, Bifidobacterium, Streptococcus* and *Enterrococcus* (Sahota *et al.,* 2008).

Toddy is a popular drink in Asian countries and is produced from fermented sap of Coconut palm (*Cocos nucifera*). It is a natural fermented food that have microorganisms that are probiotic in nature. These probiotic microorganisms have the ability to act as antimicrobial agent so it can be used as natural food additive in order to increase immunity without any side effects (Ashraf and Shah, 2011). The microorganisms on toddy fermentation produce lactic acid and CO_2 that make the toddy anaerobic and leaven the product.

Kishk is a popular fermented milk-cereal based food normally made from low-fat yoghurt or buttermilk. It is commonly called as *kishk, Kushuk, Keshkeh or Kichk* in the rural regions located between the Middle East and the Indian sub-continent. Traditionally, skimmed milk or buttermilk from churned fermented milk is mixed with cereal (*Burghol,* prepared from parboiled cracked wheat) in the ratio between 1:2 and 1:4, sun dried and ground to a powder (Tamime *et al.,* 2000). In Egypt, principal microorganisms reported in *kishk* are the heterofermentative lactobacilli i.e. *lactobacillus brevis* and the homofermentative *lactobacillus casei* and *L. palntarum* (Gaden *et al.*, 1992). Dried powder can further be reconstituted with water and simmered the mix gently over fire.

Akpan is yoghurt like beverage consumed commonly in Benin, is prepared from a partially cooked maize gruel, commonly called *Ogi*. It is usually mixed with condensed milk, ice and sugar just before consumption (Pallet, 2011).

Credible scientific research indicates many potential health foods from cereals and milk. Nutrients present in milk are essential for the well-being of people of all age groups. But in lack in availability of milk-cereal based foods in the market place and lack of consumer awareness towards the health benefits limits their consumption. Hence, cereal-milk based probiotic foods must be promoted as they can be used as a tool in addressing the problems of malnutrition and hunger. Both the major traditional drinks/foods with probiotic strains are discussed in Table 6.

Recent developments in Synbiotic Drinks

Nowadays, a considerable research focused on the development of probiotic drinks from new sources such as cereals, fruits and vegetables which serve as suitable substrates for the growth of probiotic Lactic acid bacteria with improved functionality of colonic strains because of the presence of non-digestible components as β-glucan, arabinoxylan, oligosachharides, resistant starch in cereals and glucose, fructose, hemicelluloses and dietary fibres in fruits and vegetables, which act as prebiotics. These prebiotics further serve as stimulators for the growth of useful bacteria.

Cereal based synbiotic drinks

A study on development of non-dairy probiotic drink utilizing sprouted cereals, legume and soymilk was conducted by Mridula and Sharma (2014). Non-dairy probiotic drink (PD) was developed utilizing sprouted wheat, barley, pearl millet and green gram separately with oat, stabilizer and sugar using *Lactobacillus acidophilus*-NCDC14; with soymilk and distilled water as liquid portion. Probiotic count in the final products ranged from 9.10 to 11.06, 10.36 to 11.17, 10.36 to 11.51 and 10.36 to 11.32 log cfu/mL in wheat, barley, pearl millet and green gram based PD samples, respectively, which increased with increasing level of grain flour. All the four PD with soymilk received higher sensory acceptability scores. on the basis of sensory acceptability score >7 and good probiotic count; wheat, barley and green gram at 6 g while pearl millet at 4 g level with 50:50 mL of soymilk and distilled water, were considered suitable for development of non-dairy PD.

In another study, wheat based probiotic beverage was developed using *Lactobacillus acidophilus* with proportion of different ingredients viz. sprouted wheat flour, sprouted wheat bran, oat and stabilizer using response surface methodology. Acidity, pH and probiotic count of samples prepared with *L. acidophilus* NCDC-14 was higher than that of *L. acidophilus* NCDC-16 culture and hence was found more compatible than others. Acidity (in terms of lactic acid), pH and probiotic count ranged from 0.21 to 0.45 %, 4.0 to 4.9, and 8.30 to 10.95 $\log_{10}$ cfu/ml^{-1}, respectively. An increase in Probiotic count with increasing amount of sprouted wheat and oat took place. Optimized levels for sprouted wheat flour, oat, wheat bran and guar gum were 7.86, 5.42, 1.42 and 0.6 g, respectively per 100 mL of water. Optimized probiotic beverage contained 13.19 % total solids, 1.19 % protein, 0.33 % fat, 0.10 % ash, 0.42 % crude fibre, 1.45 mg iron, calcium 15.74 mg, 11.56 % carbohydrates, 54 kcal calories and 10.43 $\log_{10}$ cfu/ml^{-1} probiotic count (Sharma *et al.,* 2013).

Another cereal based probiotic beverages using the rice and millets were prepared by Hassan *et al.* (2012). In this study, rice and millet grains were fermented with ABT-2 starter culture (*S. thermophilus,* L. *acidophilus* and *Bifidobacterium* BB-12 to obtain probiotic beverages, fortified with pumpkin and sesame seed milk. Other factors used were rice, millets, honey and the fermentation was carried out for 16 hours. The viable cell counts reached at the end of fermentation time were about 4.3×10^9 cfu/ml. Changes were observed in acidity and counts of probiotic bacteria as a result of fortification with pumpkin and sesame milk at 10%. Fermentation with ABT-2 starter culture improved the color, flavor, texture and overall acceptability of the beverages. The shelf-life of the rice and millet fermented beverages was estimated to be 15 days under refrigerated storage.

Gupta *et al.* (2010) developed a functional beverage based on lactic acid fermentation of oats using Box–Behnken design to optimize three different levels of oat, sucrose and starter culture (*Lactobacillus plantarum*). A second-order polynomial response surface equation showed the effect of the studied variables on *L. plantarum* growth. Contour maps generated using the response surface equation showed that the experimental variables significantly affected the growth of the *L. plantarum*. The optimized factors (5.5% oats, 1.25% sugar and 5% inoculum) were then, applied to prepare a fermented drink to obtain a growth of 10.4 log cfu/mL. The shelf-life of the fermented drink was monitored over a period of 21 days. β-Glucan level remain unchanged during the fermentation and also during the entire storage period.

A study was conducted by Angleov *et al.,* (2006) to combine the health benefits of probiotic culture with the oat beta-glucan. The levels of several factors, such as starter culture (lactic acid bacteria) concentration, oat flour and sucrose content, affecting the fermentation process, were established for completing a controlled fermentation for 8 h. At the end of the process, it was

Table 6: Traditional drinks/Foods from different regions of the world

Name of Product	Raw Material	Starter culture	Region/ Country	Microorganisms reported	Reference
Kaanji	Black Carrot	Natural Microflora/ Spontaneous fermentation	Punjab, India	*Lactobacillus sp, Bifidobacterium sp, Streptococcus sp, Enterococcus sp.*	Sahota *et al.*,2008
Lugri	Rice	*Phab*	Himachal, India	*S. cerevisiae, Leuconostoc sp., Lactobacillus sp.*	Thakur *et al.*, 2004
Sur	Finger millet, jiggery	*Dhaeli*	Himachal, India	*S. cerevisiae, Lactobacillus sp.*	Kumar, 2013
Boza	Wheat, rye, millet, maize and sugars	Natural Microflora/ Spontaneous fermentation	Bulgaria, Albania	*Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus coprophilus*, *Leuconostocreffinolactis*, *Leuconostocmesenteroides*and *Lactobacillus brevis*. **Yeasts** *Saccharomyces cerevisiae*, *Candida tropicalis*, *Candida glabrata*, *Geotrichum penicillatum* *Geotrichum candidum*	Gotcheva *et al.*,2000; Blandino *et al.*, 2003
Bushera	Soyabean, millets	Natural Microflora/ Spontaneous fermentation	Western highlands, Uganda	*Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Streptococcus, Lactobacillus brevis.*	Muianja *et al.*, 2003
Mahewu	Maize, Sorghum or millet malt, wheat flour	Natural Microflora/ Spontaneous fermentation	Africa and Arabian Gulf countries	*Lactococcus lactis* subsp. *lactis*	Blandino, 2003
Pozol	Maize	Spontaneous fermentation in banana leaves	Southeast ern Mexico	Molds Yeasts Bacteria	Blandino, 2003
Bhattejaanr	Rice	Murcha	India, Sikkim	*Hansenula anomala, Mucor rouxianus*	Rai 2006; Blandino 2003
Ang-kak (anka, red rice)	Rice	Monascus spp	China, Southeast Asia, Syria	*Monascus purpureus*	Han *et al.*, 2001
Sufu	Soybean		China	*Actinomucor spp., Mucor spp., Rhizopus spp.*	Han *et al.*, 2001

(Cont...)

Togwa	sorghum, maize and millet	Spontaneous fermentation	Tanzania	*Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus cellobiosus, Weissella confusa, Pediococcus pentosaceus, Lactobacillus plantarum.* Yeasts *Saccharomyces cerevisiae,* *Candida tropicalis,* *Candida pelliculosa.*	Mugla *et al.*, 2003
Kvass	Rye barley malt and sucrose	Baker's yeast or back-slopping	Eastern Europe	Lactobacillus casei, L. mesenteroides. **Yeasts** S. cerevisiae	Nyanzi and Jooste, 2012

observed that viable cell counts reached about 7.5 10^{10} cfu/ml. Beta-glucan content in the drink (0.31-0.36%) remained unchanged both throughout fermentation and storage of the drink. The shelf life of the oat drink was estimated to 21 days under refrigerated storage.

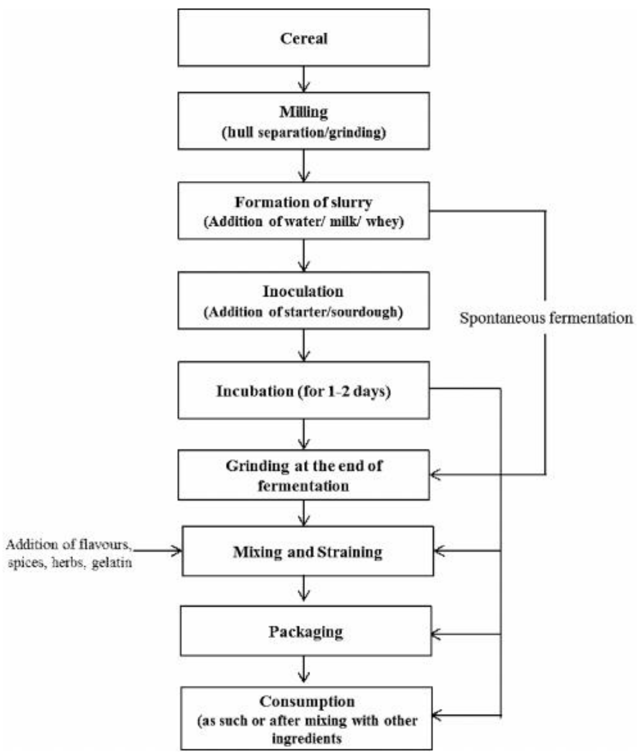

Fig. 2: An outlined process for the preparation of cereal based synbiotic drinks

A yogurt-like oat based product ''Oat bio Lacto'' containing oat beta-glucan, oat lipid unsaturated fatty acids and living probiotic lactic acid bacteria that provided polyfunctional nutrition was developed by Bekers *et al.* (2001). This functional bio-active food product was developed using commercial rolled oats and fat-free milk as raw materials. Enzymatic hydrolysis of oat mash was achieved using α-amylase the substrate was then cultivated with different lactic acid bacterial strains *viz., Lactobacillus acidophilus, Bifidobacterium* sp. and commercial starter cultures ABT-1. A fat-free milk additive stabilized the texture of the oat mash and caused an increase in protein content of the final product whereas improved taste properties were imparted by *Bifidobacterium* sp.

Mårtensson *et al.* (2001) prepared a fermented, non-dairy product based on oats using a new kind of oat base, Adavena M40, a concentrated liquid (with a dry matter content of 16 or 18 percent) derived entirely from oats, with maltose as the main carbohydrate source and an intact β-glucan content. It was fermented with two different yoghurt cultures. The oat base was heat treated for 5 min at 85^{O}C, prior to inoculation. Addition of xanthan gum (0.03 percent w v^{-1}) improved the texture and overall appearance of the product. The oat-based, yoghurt-like product showed high acceptability in terms of acidity, texture and overall appearance. Addition of flavours resulted in a higher acceptance of the final products. The β-glucan content was still high after the fermentation process.

Santos (2001) developed a probiotic beverage with the fermented cassava flour using mixed culture of *Lactobacillus plantarum*, which were amylolytic strains of *Lactobacillus casei Shirota* and *Lactobacillus acidophilus*. The best parameters of the fermentation were 8% inoculation rate, incubation temperature and period as 35^{O}C and 16 h, respectively, and 20% of cassava flour. At the end of the fermentation, the amount of bacteria was 2.8 × 10^9 cells/ml of lactic amylolytic bacteria and 2.3 x 10^9 cells/ml of probiotic bacteria. The final lactic beverage had 36% of guava juice, 10% of sugar and 54% of fermented lactic beverage. The lactic beverage maintained its microbiological and physico-chemical quality for 28 days storage period at 4^{O}C.

Fruit based Synbiotic Drinks

The fruit juices have been suggested as an ideal medium for the functional health ingredients as they inherently contain beneficial nutrients, they have taste profiles that are pleasing to all the age groups, and because they are perceived as being healthy and refreshing (Tuorila and Cardello, 2002). Fruits are the natural source of sugars, flavours, vitamins, dietary fibers, minerals, antioxidants, and do not contain any dairy allergens that might prevent usage by certain segments of the population (Luckow and Delahunty, 2004). The incorporation of the fruits in the dairy products increases their acceptability and also adds to the variety. A number of fruits have been incorporated in the various fermented products and some of the literature is reviewed here. A general out line of the process for the preparation fruit based synbiotic is depicted in Figure 3.

A cashew apple juice probiotic drink containing *Lactobacillus casei* was developed by Pereira *et al.,*(2011). The optimum conditions for probiotic cashew apple juice production were initial pH 6.4, fermentation temperature of $30^{O}C$, inoculum size of 7.48 Log cfu/ml and 16 h of fermentation process. It was observed that the *Lactobacillus casei* grew during the refrigerated storage also. Viable cell counts were higher than 8.00 Log cfu/ml during storage period of 42 days.

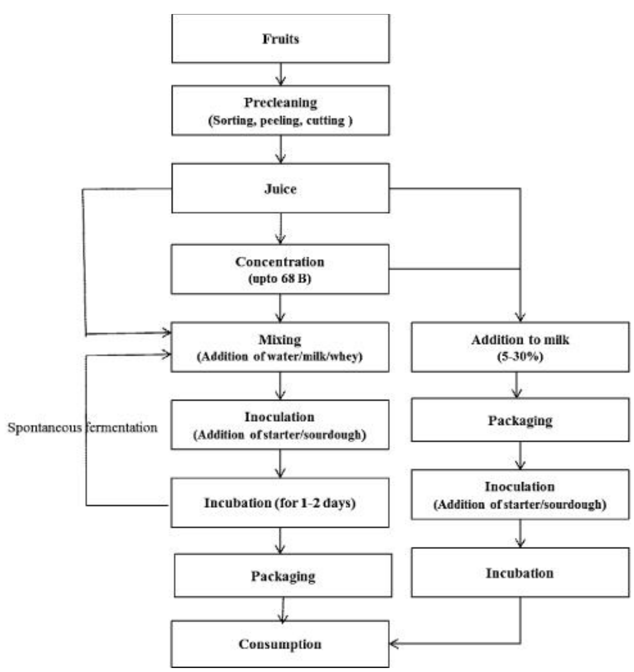

Fig. 3: An outlined process for the preparation of fruit based synbiotic drinks

The fermented juice with *Lactobacillus casei* is a good and healthy alternative functional food containing probiotics. Cashew apple juice showed to be as efficient as dairy products for growth of *Lactobacillus casei*.

Rakin *et al.* (2007) enriched beetroot and carrot juices with the brewer's yeast autolysate before lactic acid fermentation with *Lactobacillus acidophilus*. The addition of the autolysate the increased the number of lactic acid bacteria during the fermentation, reduction of the time of fermentation and enrichment of the vegetable juices with amino acids, vitamins, minerals and antioxidants. The use of spent brewer's yeast from the brewery was important for the economic optimization of the fermentation. A mixture of beetroot and carrot juices had the optimum proportions of pigments, vitamins and minerals (Rakin *et al.* 2007).

Hardaliye is a lactic acid fermented beverage produced from the natural fermentation of the red grape, or grape juice with the addition of the crushed mustard seeds and benzoic acid. This beverage is well-known and found in the Thrace region of Turkey. It has been produced and consumed since ancient times. The mustard seed's eteric oils affect the yeasts and also give flavor to the final product. Benzoic acid inhibits, or decreases alcohol production by affecting the growth of yeast. Once fermented, the *hardaliye* is stored at a temperature of $4^{O}C$ and consumed either fresh, or aged. The lactic acid bacteria found in the beverage were; *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus casei* subsp. *Pseudoplantarum*, *Lactobacillus brevis*, *Lactobacillus pontis*, *Lactobacillus acetotolerans*, *Lactobacillus sanfransisco* and *Lactobacillus vaccine stercus*. This characterization allowed the selection of appropriate strains for the manufacture of *hardaliye* using pasteurized or sterile filtered grape juices (Arici and Coskun, 2001).

Yousef *et al.,* (2013) studied the effect of fruit pulp on yoghurt quality. that was prepared with different fruit pulps including apple, banana and strawberry and stored up to 10 days. The fruit were added at the rate of 7% and 10% (w/w). Significant differences between plain yogurt and fruit yogurt in the pH, moisture, ash, protein, carbohydrate content and titratable acidity was found. The results showed that acidity increased over the storage period. After 10 days storage, highest values for water holding capacity (WHC) (90.32%) and lowest values for syneresis (12%) were observed in yogurt with 10 % banana pups. The yoghurt containing 10% strawberry pup had higher acidity and syneresis than other fruit yogurts. Sensory evaluation results showed that there were no significant differences among the yoghurt samples. The yoghurt containing strawberry had the highest overall acceptability scores as compared to other fruit yoghurt samples.

Fruit yoghurt with different level of fruit juice of different fruits (strawberry, orange and grape) was also prepared and studied (Hossain *et al.* 2012). It was observed that the smell, taste, body, consistency, color and texture of all fruit yoghurts were equally acceptable. Statistical analysis however, showed that yoghurt with 10% orange juice was more acceptable than others, comparing all quality characteristics. Overall an increase in moisture content and acidity of fruit yoghurts were observed than the plain yoghurt. The fat, protein, carbohydrates and ash content of strawberry and orange fruit yoghurt decreased than normal plain yoghurt. But the carbohydrates content of grape yoghurt increased because grape contains more sugar than milk and other two fruits. The microbiological quality of the fruit yoghurts was also acceptable because of acid content of the fruits. In case of strawberry yoghurt, fruit juice concentration more than 5% was not suitable for fruit yoghurt because it was highly acidic and curd was cracked at refrigerated condition.

Fruit-milk-soya based probiotic drinks

Effect of yoghurt cultures and probiotic cultures on physico-chemical and sensory properties of mango soy fortified probiotic yoghurt (MSFPY) was studied by Shilpi and Kumar (2013). Mango soy fortified probiotic yoghurt (MSFPY) was prepared by using blends of 78.3% toned milk, 14.5% soy milk and 7.2% mango pulp. Effect of yoghurt cultures *Streptococcus thermophilus* (ST), *Lactobacillus bulgaricus* (LB) and probiotic culture *Bifidobacterium bifidus* (BB), *Lactobacillus acidophilus* (LA) on physico-chemical properties and sensory properties were studied using different inoculum levels as per response surface methodology (RSM). The optimized concentration of cultures was found to be 1.75%, 1.95%, 2.44% and 1.37% for ST, LB, BB and LA respectively which yielded an acceptable quality of MSFPY having acidity 0.73%, total solids 14.02%, syneresis 14.12% and scored 8.5 on hedonic rating.

Dalev *et al.* (2006) prepared four fermented probiotic beverages fermented with selected probiotic strains of *Bifidobacterium breve*, *B. infantis*, *B. animalis/lactis*, *Lactobacillus plantarum*, *L. casei* and *Streptococcus thermophilus* were prepared. During the study a mixture of cheese whey and soy preparation, and one on a soy preparation were used. After fermentation, the beverages were supplemented with processed fruits, and subsequently, their sensory properties were evaluated by Quantitative Descriptive Analysis (QDA). Eighteen attributes for the investigated products, describing odour, appearance, taste, and consistency, were defined. The results obtained showed that during fermentation and subsequent fruit supplementation non-desired attributes of the soy preparation, like beany taste and flavour as well as aftertaste disappeared, and pleasant fruity, slightly acidic and sweet tastes appeared. All the beverages were appraised with relatively high overall score (7.07–7.82 out of 10) over the control non-fermented soy preparation (score 1.13 out of 10). The final products, as a new probiotic beverages, were characterized with good sensory properties, and in addition a high cell number of the probiotic bacteria (about 10^9 cells/mL).

Impact of synbiotic foods on health

The principal aim of adding prebiotics/probiotics or synbiotics to the diet is to beneficially affect the consumer by improving the intestinal microflora balance that might lead to improved nutrition and health (Roberfroid, 2004). Studies conducted have proved the efficacy of synbiotic foods over probiotics. The effects of probiotics (*Bifidobacterium animalis* subsp. *Lactis* HN019 and *Lactobacillus rhamnosus* HN001) and prebiotics (fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS), and inulin) individually and in synbiotic combinations (*B. lactis* HN019+FOS, *B. lactis*HN019+GOS, *B. lactis* HN019+inulin, *L. rhamnosus* HN001+FOS, *L. rhamnosus* HN001+GOS, and *L. rhamnosus* HN001+inulin) on large bowel health were investigated in rats fed with the respective diets for 21 days. All experimental treatments led to significantly lower body weight gains and decreased caecal acetic acid concentrations compared to the control diet (no pro, pre and synbiotics). Caecal *Bifidobacterium* spp. or *Lachno spiraceae* were increased in *L. rhamnosus* HN001, FOS or inulin treatments. Rats fed *L. rhamnosus* HN001 had enhanced colonic α-defensin 1and (MUC)-4 gene expression. All synbiotic combinations increased the mucin (MUC4) gene expression (Paturi *et al.,* 2015).

In another study conducted in 2013, 120 outpatients with ulcerative colitis were randomly sorted into three groups of 40 patients each for probiotic, prebiotic, or synbiotic therapy. The probiotic group ingested one daily capsule consisting of *Bifidobacterium longum* 2×10^9 colony forming units and the prebiotic group ingested daily 8.0g doses of psyllium. The synbiotic group underwent both treatments. All patients completed Inflammatory Bowel Disease Questionnaires at the onset of the trial, at the 2 wkmidpoint, and at the 4 wkend of the trial. Individual scores improved as probiotics, emotional function (P = 0.03); prebiotics, bowel function (P = 0.04) and synbiotics, systemic and social functions (P = 0.008 and P = 0.02). The protein decreased significantly only with synbiotic therapy (from 0.59 to

0.14 mg/dL, P = 0.04). No adverse events were reported. Hence it was concluded that patients with ulcerative colitis on synbiotic therapy experienced greater quality of life changes than patients on probiotic or prebiotic treatment (Fujimori *et al.,* 2009). Similar results were reported in another study by using synbiotic drink with probiotic strain of *Bifidobacterium* and *Fucus evanescens* polysaccharides with prebiotic activity and broad spectrum of the biological action on the patients with chronic diseases of the gastrointestinal tract and concominant intestinal dysbacteriosis (Kuznetsova, *et al.,* 2013).

Future Thrust

Although many companies have launched their probiotic foods and beverages in the Indian market since last few years yet these foods are not very popular among the rural people due to lack of knowledge and their higher market price. Many traditional foods have potential to behave as a synbiotic as they are known to contain both probiotic and prebiotic factors. The advantages with the indigenous traditional foods are that these are well accepted by the people and they can be stored at room temperature for a longer period of time. However, the poor hygienic conditions at production level leads to the uncertain microflora in the final food which questions the reliability of the final product. The production of such foods using indigenous raw-material along with modern technical methods will ensure the reach of these products to the common masses at a cheaper rate. Another challenge in this sector is that most of the probiotic foods in the markets worldwide are milk based. The milk based probiotic drinks are not suitable for subjects with lactose intolerance or milk protein allergies (Prado *et al.,* 2008).

Furthermore, many researchers have found benefits of synbiotic drinks over the probiotic drinks. Future researches must focus on the development of synbiotic drinks with composite substrates. By reviewing the various aspects discussed here it is concluded that synbiotic foods can offer a cheaper substrate, huge potential for development of new exotic tastes, cheaper prices and more health benefits as compared to the probiotic drinks.

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