CHAPTER -2

REVIEW OF LITERATURE

The current research study is related to a fermented product development aims to combine the potential health benefits of the good bacteria with their ability to grow in with cereals, resulting in a nutritionally healthy and desirable product for the consumers. The present chapter also intends to measure the greatness and to open up new lines of present and imminent aspects of our investigation entitled "Probiotic profiling and organoleptic evaluation of cereal buttermilk based fermented drink and its market potential".

Following flow will be followed to read the collected literature about the present research:

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- 2.6.1 Prebiotics in cereal based beverages

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2.1 Overview of beverage industry

The beverage industry consists of two major categories and eight sub-groups. The non-alcoholic category is comprised of soft drink syrup manufacture; soft drink and water bottling and canning; fruit juices bottling, canning and boxing; the coffee industry and the tea industry. Alcoholic beverage categories include distilled spirits, wine and brewing.

Although many of these beverages, including beer, wine and tea, have been around for thousands of years, the industry has developed only over the past few centuries.

The beverage products industry, viewed as an aggregate group, is highly fragmented. This is evident by the number of manufacturers, methods of packaging, production processes and final products. The soft drink industry is the exception to the rule, as it is quite concentrated. Although the beverage industry is fragmented, ongoing consolidation since the 1970s is changing that.

2.2 Consumption pattern of beverages worldwide

With urbanisation and exposure to the West, Indian consumers today are increasingly becoming conscious of their lifestyle including food and beverage choices, in some cases prompting a shift from colas to healthier options such as juices, milk-based flavoured beverages, etc.

"Buying behaviour is changing. India is the diabetic capital of the world and there is much more awareness now leading to growth of fruit-based drinks in the last one year," says Abneesh Roy, SVP institutional equities, Edelweiss Securities. "In the medium-to long-term, fruit-based and milk-based beverages will take away a lot of market share from colas with ready-to-drink beverages in the dairy segment also coming to the fore." The market share of fruit juices, nectars and juice drinks stands at around 25.1%, which is still less than that of carbonated drinks (approx 47.9%), as per KPMG (Thomson, 2012).

India's carbonated drinks industry, estimated at over Rs.14,000 crore (media reports), is dominated by Coca-Cola and Pepsi. But these companies too are being forced to relook at their product mix. While Coca-Cola is exploring the launch of coconut water and flavoured milk, a case in point would be 7UP with 30% less sugar which was recently introduced in Gujarat. Another example is 7UP Nimbooz Masala Soda, a carbonated beverage with 5% lemon juice, introduced in 2013. Arvind Singhal, MD, Technopak agrees that there is pressure on the topline of cola companies, and the consumption of juices is significantly increasing when the consumer is on the go (Poranki and Kameswara, 2016).

PepsiCo recently launched a new sub-category Tropicana Essentials, of which the first variant is 'Fruits & Veggies' catering to the millennial. Tropicana aims to continue driving local sourcing with juices like Mosambi, Guava and Pomegranate with its Delights range (Pepsi Co Annual Report, 2017). Sensing similar consumer trends, milk-based beverages are also coming to the fore with Amul actively pushing its Kool range, Danone Nutricia launching Protinex Grow for kids aged between 8-15, while Nestlé India has relaunched Milo as a ready-to-drink with less than 10 grams of added sugar per serve in a 180ml pack. Arvind Bhandari, GM — dairy, Nestlé India says, "The introduction is part of our vision to provide dairy-based beverage solutions rooted in nutrition, health and wellness" (Nestlé India Limited Annual Report, 2017).

2.3 Consumer demand for health beverages

The health beverage industry is emerging on the back of shift in consumer preference due to changing lifestyle. The fortification of health beverage, ranging from ingredients such as herbs, vitamins, minerals and amino acids to additional raw fruits and vegetables, is expected to grow at a CAGR (Compound Annual Growth Rate) of around 20% during the period of 2016 to 2022 as per RNCOS analysis. While highlighting the potential of this industry, Shushmul Maheshwari, CEO – RNCOS said, “The functional beverages market offers new product opportunities to those players that understand consumers’ preferences and can develop and market functional beverages that meet consumers’ expectations.” According to RNCOS, Latest Analysis Reports the functional drinks are non-alcoholic drinks that hydrates the body and provide overall nutritional well-being. The Indian market is evolving with the growing health awareness. The new generation is ready to pay a premium price for functional advantage from such health beverage products. Rising knowledge about wellness and health along with inclining expenditure on beverages has increased the number of consumers accepting such beverages. The increasing health consciousness amongst Indian populace is acting as a strongest pull toward the health beverages industry. Health beverages are products with ingredients that are added for specific health benefits that are beyond basic nutrition. While Indian consumers are becoming increasingly aware of health and nutrition, rising incomes mean that they can also splurge on the products with attributes that they value. The attributes that Indian consumers seek are immune or energy boosting, nutrients and vitamin lost during workout etc.

2.4 Fermentation as a technology in health beverages

2.4.1 History of fermentation

The term "fermentation" comes from latin word fervere meaning "to boil". Fermentation is defined as a form of energy-yielding process from an organic substrate, usually carbohydrate, without the involvement of an exogenous oxidizing agent (Bourdichon *et al.*, 2012). This process usually occurrs in the absence of oxygen and uses organic molecules as electron acceptors. It is a desirable process that is mediated by microorganisms and their enzymes that causes a biochemical modification of primary food products (Liu *et al*.,2011).

Food fermentation is the oldest method of food preservation and originated several million years ago. The records of conversion of barley to beer date back to more than 5000 years (Borgstrom, 1968). Preservation of food by drying and fermentation were the main techniques used before the discovery of canning and freezing. It is relatively efficient, low energy consuming process which needs no refrigeration or other food preservation technologies and also increases the shelf life of foods. Fermentation ensures not only increased shelf life and microbiological safety of food but may also make some foods more digestible and lower or eliminate the toxicity of substrate like cassava (Caplice and Fitzgerald, 1999). Food fermentation is important in developing countries where the lack of resources limits the use of techniques such as vitamin enrichment of foods, and the use of energy and capital-intensive processes for food preservation (Parveen and Hafiz, 2003). This method of food preservation is of economic importance in the areas where due to lack of resources, the facilities such as cold storage and drying are not available.

The first fermentation known to mankind was alcoholic fermentation. It is believed that the discovery of food fermentation was purely by chance and the reports about the origin of fermented foods are lacking. Anthropologists have suggested that it was the production of alcohol which motivated primitive people to settle down and start agriculture for survival (Thrall, 2010). There is reliable information about the production of fermented drinks over 7000 years ago in Babylon, 5000 years ago in Egypt, 4000 years ago in Mexico and 3500 years ago in Sudan (Romero-Luna *et al*, 2017; Mahgoub, 2018). There are reference of fermented dairy products in some of the earliest writings of civilization such as Bible and sacred books of Hinduism (Ray *et al*, 2014). The origin of bread making is probably in Egypt over 3500 years ago (Rabie *et al*, 2011). In ancient tombs several triangular loaves of bread have been found. Egyptians had over 50 different types of cakes, unleavened breads and bread leavened with beer foam or sourdoughs. There is evidence of fermented meat products being produced for king Nebuchadnezzar of Babylon (Chilton *et al*, 2015).

Asians (Indonesians) were the pioneers in fermentation of vegetables to generate meat like flavours and textures. Koreans are credited for the production of acid fermented vegetables while Indians discovered methods for souring and leavening cereal-legume batters (Rhee *et al*, 2011). Fermentation technologies play an important role in ensuring the food security of millions of people around the world particularly marginalized and vulnerable groups (Franz *et al*, 2014). The food fermentation technology utilizes the ability of growth of microorganisms on various substrates for the production of fermented foods (milk, cereals, legumes, meat, fish, etc.), a variety of beverages and fermented vegetable pickles e.g. bread, beer, cheese, idli, tempeh, miso, sauerkraut, fermented sausages, etc (Soni and Dey, 2014).

2.4.2 Fermentation process

A batch fermentation can be considered to be a closed system. At time t=0 the sterilized nutrient solution in the fermentor is inoculated with microorganisms and incubation is allowed to proceed. In the course of the entire fermentation, nothing is added, except oxygen (in case of aerobic microorganisms), an antifoam agent, and acid or base to control the pH. The composition of the culture medium, the biomass concentration, and the metabolite concentration generally change constantly as a result of the metabolism of the cells. After the inoculation of a sterile nutrient solution with microorganisms and cultivation under physiological conditions, four typical phases of growth are observed (Paulová *et al*, 2013; Gutiérrez *et al*, 2010) as indicated in Fig 2.1

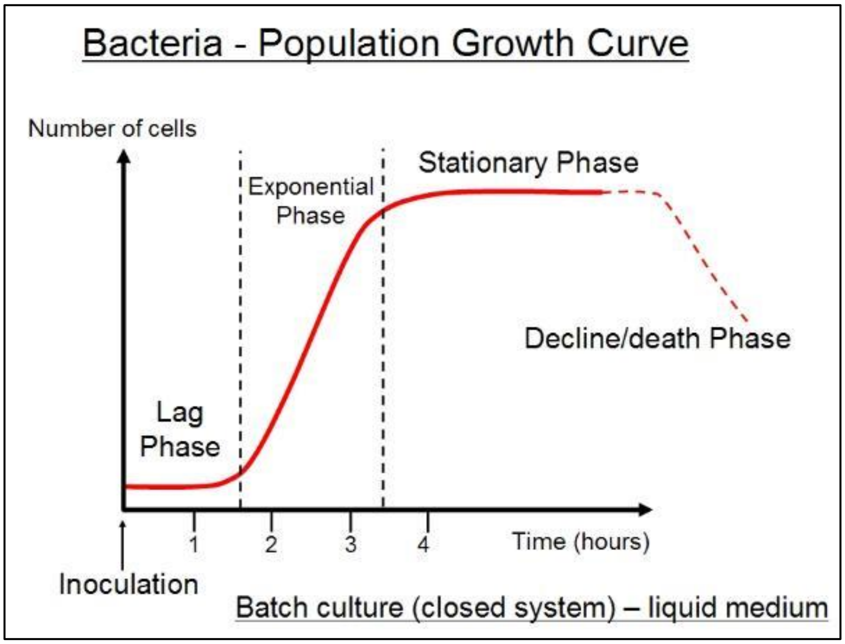

Fig 2.1 Growth curve of a bacterial culture

2.4.2.1 Lag phase

Physicochemical equilibration between microorganism and the environment following inoculation with very little growth (Paulová *et al*, 2013; Gutiérrez *et al*, 2010).

2.4.2.2 Log phase

By the end of the lag phase cells have adapted to the new conditions of growth. Growth of the cell mass can now be described quantitatively as a doubling of cell number per unit time for bacteria and yeast's, or a doubling of biomass per unit time for filamentous organisms as fungi. By plotting the number of cells or biomass against time on a semilogarithmic graph, a straight line results, hence the term log phase. Although the cells alter the medium through uptake of substrates and excretion of metabolic products, the growth rate remains constant during the log phase. Growth rate is independent of substrate concentration as long as excess substrate is present (Paulová *et al*, 2013; Gutiérrez *et al*, 2010).

2.4.2.3 Stationary phase

As soon as the substrate is metabolized or toxic substances have been formed, growth slows down or is completely stopped. The biomass increases only gradually or remains constant during this stationary phase, although the composition of the cells may change. Due to lysis, new substrates are released which then may serve as energy sources for the slow growth of survivors. The various metabolites formed in the stationary phase are often of great biotechnological interest (Paulová *et al*, 2013; Gutiérrez *et al*, 2010).

2.4.2.4 Death phase

In this phase the energy reserves of the cells are exhausted. A straight line may be obtained when a semilogarithmic plot is made of survivors versus time, indicating that the cells are dying at an exponential rate. The length of time between the stationary phase and the death phase is dependent on the microorganism and the process used. The fermentation is usually interrupted at the end of the log phase or before the death phase begins (Paulová *et al,* 2013; Gutiérrez *et al,* 2010).

2.4.3 Physiochemical changes during cereal fermentation

Natural fermentation of cereals leads to a decrease in the level of carbohydrates as well as some non-digestible poly and oligosaccharides. Certain amino acids may be synthesised and the availability of B group vitamins may be improved. Fermentation also provides optimum pH conditions for enzymatic degradation of phytate which is present in cereals in the form of complexes with polivalent cations such as iron, zinc, calcium, magnesium and proteins. Such a reduction in phytate may increase the amount of soluble iron, zinc and calcium several folds. (Kohajdová and Karovičová, 2007)

Fermentation also leads to a general improvement in the shelf life, texture, taste and aroma of the final product. During cereal fermentations several volatile compounds are formed, which contribute to a complex blend of flavours in the products (Martínez, 2012).

Traditional fermented foods prepared from most common types of cereals (such as rice, wheat, corn or sorghum) are well known in many parts of the world (Sandhu *et al*, 2017). Some are utilixed as colorants, spices, beverages and breakfast or light meal foods, while a few of them are used as main foods in the diet. The microbiology of many of these products is quite complex and not known. In most of these products the fermentation is natural and involves mixed cultures of yeasts, bacteria and fungi (Bokulich *et al*, 2014). Some microorganisms may participate in parallel, while others act in a sequential manner with a changing dominant flora during the course of the fermentation. The common fermenting bacteria are species of Leuconostoc, Lactobacillus, Streptococcus, Pediococcus, Micrococcus and Bacillus. The fungi genera spergillus, Paecilomyces, Cladosporium, Fusarium, Penicillium and Trichothecium are the most frequently found in certain products. The common fermenting yeasts are species of Saccharomyces, which results in alcoholic fermentation (Azam *et al*, 2017)

The type of bacterial flora developed in each fermented food depends on the water activity, pH, salt concentration, temperature and the composition of the food matrix. Most fermented foods, including the major products that are common in the western world, as well of many of those form other sources that are less well characterised, are dependent on lactic acid bacteria (LAB) to mediate the fermentation process (Nyambane *et al*, 2014). Lactic acid fermentation contributes towards the safety, nutritional value, shelf life and acceptability of a wide range of cereal based foods (Nwachukwu *et al*, 2018). In many of those processes, cereal grains, after cleaning, are soaked in water for a few days during which a succession of naturally occurring microorganisms will result in a population dominated by LAB. In such fermentations endogenous grain amylases generate fermentable sugars that serve as a source of energy for the lactic acid bacteria. Fermentation is often just one step in the process of fermented food preparation. Other operations such as sise reduction, salting or heating also affect the final product properties (Magala *et al*, 2015).

The preservative role of lactic fermentation technology has been confirmed in some cereal products. The antibiosis mediated by LAB has been attributed to the production of acids, hydrogen peroxide and antibiotics.

2.4.4 Functional properties of micro organisms in fermented foods

Existing scientific data show many fermented foods have both nutritive and non-nutritive components in foods, which have the potential to modulate specific target functions in the body relevant to well-being and health of the consumers. However, 90% of naturally fermented foods and alcoholic beverages in different countries and regions of the world are still at home production under traditional conditions. Naturally fermented foods and beverages contain both functional and non-functional microorganisms (Tamang *et al*., 2016).

Functional microorganisms transform the chemical constituents of raw materials of plant/animal sources during food fermentation thereby enhancing the bio-availability of nutrients, enriching sensory quality of the food, imparting bio-preservative effects and improvement of food safety, degrading toxic components and anti-nutritive factors, producing antioxidant and antimicrobial compounds, stimulating the probiotic functions, and fortifying with some health-promoting bioactive compounds (Tamang *et al*., 2009, 2016; Bourdichon *et al*., 2012; Thapa and Tamang, 2015). Among bacteria associated with fermented foods and alcoholic beverages, lactic acid bacteria (LAB) mostly species of Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Weissella, etc. are widely present in many fermented foods and beverages (Axelsson *et al*., 2012; Holzapfel and Wood, 2014).

Functional properties of microorganisms in fermented foods include probiotics properties (Hill *et al*., 2014), antimicrobial properties (Meira *et al*., 2012), antioxidant (Perna *et al*., 2013), peptide production (De Mejia and Dia, 2010), fibrinolytic activity (Kotb, 2012), poly-glutamic acid (Chettri and Tamang, 2014), degradation of antinutritive compounds (Babalola, 2014), etc. which may be important criteria for selection of starter culture(s) to be used in the manufacture of functional foods (Badis *et al*., 2004).

2.4.4.1 Probiotic Microorganisms

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill *et al*., 2014). The most commonly used probiotic bacteria belong to the heterogeneous group of LAB (Lactobacillus, Enterococcus) and to the genus Bifidobacterium, however, yeasts and other microbes have also been developed as potential probiotics during recent years (Ouwehand *et al*., 2002). Products containing probiotic bacteria generally include foods and supplements (Varankovich *et al*., 2015). Fermented milk products are the most traditional source of probiotic strains of lactobacilli (Bernardeau *et al*., 2006; Shah, 2015).

Fermentation-associated microorganisms might alter the intestinal composition or function of the autochthonous microbiota in the GI tract. However the magnitude of these changes and importance to probiotic efficacy is currently a point of contention (Kolmeder *et al*, 2016; Kato-kataoka *et al*, 2016). Three ways in which such changes could occur include trophic interactions (e.g. production of SCFA), direct inhibition or stimulation of competitors, and indirect effects as a result of impacting the host (Derrien and Hylckama, 2015)

Fermented milk supplemented with probiotics can improve intestinal health, humoral and cell-mediated immunity (Wang *et al*, 2012), and salivary and fecal antibodies (Surono *et al*, 2011; Campeotto *et al*, 2011). Some evidence suggests that this can lead to reduced incidence, or duration, of respiratory infections (Guillemard *et al*, 2010; Makino *et al*, 2010; De Vrese *et al*, 2006) presumably because the priming at the intestinal mucosal level impacts the lung's immune response. This is just one example of distant site health effects of probiotic fermented foods, but many others occur, including improvements to vaginal (Gardiner *et al*, 2002), bladder (Ohashi *et al*, 2002), bone (Narva *et al*, 2004), liver (Higashikawa *et al*, 2020; Moroti *et al*, 2012), body mass and blood pressure indices (Sharafedtinov *et al*, 2013) and skin health (Peguet *et al*, 2008).

2.4.4.2 Antioxidant Activity

Antioxidant activities in fermented foods include 1,1-diphenyl2-picryl hydrazyl (DPPH) radical scavenging activity, 2,20 -azinobis (3-ethylbenzo-thiazoline-6-sulfonic acid; ABTS) radical scavenging activity, total phenol content (TPC) estimation, and reducing power assay (Liu and Pan, 2010; Abubakr *et al*., 2012). Many Asian fermented soybean foods have antioxidant properties, e.g., natto, Bacillus-fermented soybean food of Japan (Ping *et al*., 2012), chungkokjang and jang, fermented soybean foods of Korea (Shin and Jeong, 2015), douchi, a fermented soybean food of China (Wang *et al*., 2007a), kinema, Bacillus-fermented soybean food of India and Nepal (Moktan *et al*., 2008), bekang and tungrymbai, Bacillus-fermented soybean foods of India (Chettri and Tamang, 2014), thua nao, Bacillus-fermented soybean food of Thailand (Dajanta *et al*., 2013), and tempe mold-fermented soybean food of Indonesia (Nurrahman *et al*., 2013). Antioxidant activities have also been observed in kimchi (Park *et al*., 2011) and yogurt (Sabeena *et al*., 2010).

2.4.4.3 Peptide Production

Bioactive peptides are formed during food fermentation by proteolytic microorganisms (De Mejia and Dia, 2010). In fermented foods peptides have some functional properties such as immunomodulatory (Qian *et al*., 2011), antithrombic (Singh *et al*., 2014), and antihypertensive properties (Phelan and Kerins, 2011). Species of Bacillus are involved in enzymatic hydrolysis of protein producing peptides and amino acids, which claim to have health benefits (Nagai and Tamang, 2010). Inhibitory properties of Angiotensin converting enzyme (ACE) have been studied in various fermented milk products such as kefir (Quiros *et al*., 2005), koumiss (Chen *et al*., 2010), yogurt (Papadimitriou *et al*., 2007), fermented camel milk (Moslehishad *et al*., 2013).

2.4.4.4 Production of Enzymes by Microorganisms

During food fermentation microorganisms produce enzymes to break down complex compounds to simple bio-molecules for several biological activities such as proteinase, amylase, mannase, cellulase, and catalase in many Asian fermented soybean foods by Bacillus spp. (Chettri and Tamang, 2014). Bacillus subtilis subsp. natto in natto produces nattokinase showing fibrinolytic activity (Mine *et al*., 2005; Kotb, 2012). Among bacteria isolated from fermented foods, B. subtilis and B. amyloliquefaciens (Chang *et al*., 2012; Zeng *et al*., 2013; Singh *et al*., 2014), Vagococcus carniphilus, V. lutrae, Enterococcus faecalis, E. faecium, E. gallinarum, and P. acidilactici (Singh *et al*., 2014), and Virgibacillus halodenitrificans SK1-3-7 isolated from fish sauce fermentation (Montriwong *et al*., 2012) produce fibrinolytic enzymes.

2.4.5 Fermented beverages global and national scenario

Fermented foods contribute to about one third of the diet worldwide (Chilton *et al*, 2015). Globally there are varieties in fermented foods and beverages and so do the ingredients and recipes for making them (Swain *et al*, 2014). Fermented beverages hold a long tradition and contribution to the nutrition of many societies and cultures worldwide. Traditional fermentation has been empirically developed in ancient times as a process of raw food preservation and at the same time production of new foods with different sensorial characteristics, such as texture, flavour and aroma, as well as nutritional value. Low-alcoholic fermented beverages (LAFB) and non-alcoholic fermented beverages (NAFB) represent a subgroup of fermented beverages that have received rather little attention by consumers and scientists alike, especially with regard to their types and traditional uses (Baschali *et al*, 2017).

Based on archaeological and archaeobotanical findings, it is generally believed that over 9000 years ago individuals of the globe were already fermenting beverages (Marshall and Mejia, 2011). For instance, remnants in jars and vessels suggest that winemaking was popular in Neolithic Egypt and Middle East (Prajapati and Niar, 2008). With regard to fermented foods in liquid form, in Western societies, beverages made with alcohol-producing yeasts, such as beers and wines, are the dominant ones. But fermentation need not always result in a beverage with alcoholic content (Sõukand *et al*, 2015). Low-alcoholic fermented beverages (LAFB) and non-alcoholic fermented beverages (NAFB) have been treasured as major dietary constituents in numerous European countries because of their keeping quality under ambient conditions and prolonged shelf-life, thereby contributing to food security and improving food safety (Standage, 2005).

Beverages developed from fermented cereals and pulses along with millets have been the staple food in Asian and African subcontinents from early times (Adebiyi *et al*, 2017). These foods are also becoming popular in developed world due to high nutritive value, organoleptic characteristics and easy digestibility.

Kefir - Kefir or kefyr (in Central Asia and Middle East) or kephir/ kiaphur/kefer/knapon/kepi/kippi (in the Balkan–Caucasian region) is one of the oldest milk-based fermented beverages. It can be made from any type of milk (goats', sheep's, cows', camel, buffalo) and kefir grains (Altay *et al*, 2013; Kabak and Dobson, 2011). Nowadays, novel varieties are also being made from milk substitutes, such as soya, rice and coconut milk. The type and amount of milk and the complex interactions between yeast and lactic acid bacteria (LAB) may influence the sensorial and textural properties of kefir. Kefir is a self-carbonated (some effervescence caused by carbon dioxide), slightly foamy and viscous beverage, with a uniform creamy and elastic consistency and sour, acidic and slightly alcoholic flavour. Kefir is regarded as an easily digested, effervescent fermented milk beverage and is esteemed for its nutritional value (Marsh *et al*, 2014; Panesar *et al*, 2011)

Ayran - Ayran is a dairy non alcoholic fermented beverage. It is a salt-containing yoghurt drink made from cows' milk or other types of milk (Altay *et al*, 2013). Ayran is consumed in Turkey (Marsh *et al*, 2014), Bulgaria, Macedonia, Kazakhstan, Kyrgyzstan and Azerbaijan. Ayran is a low-viscosity drink, easily digestible and consumed mainly during the summer months. Ayran is traditionally prepared by blending yoghurt with water (30–50 %) and salt (0·5–1 %), is produced daily and consumed fresh (homemade version). Microbiota of fermentation consists of LAB bacteria such as Lb. delbrueckii subsp. bulgaricus and S. thermophilus, with microbial populations varying due to several factors, such as the increase of the acidity (Kabak and Dobson, 2011).

Boza - Boza, a cereal-based fermented beverage, is a type of millet beer. In this respect, its origin can be traced back to 8000– 9000 years ago. The word boza derives from the Persian word buze

(Blandino *et al*, 2003), which means millet. It is made from wheat or rice semolina or from a combination of rye, oat, barley and millet flour for best quality and taste. Boza is a source of, protein, carbohydrate, fibre and vitamins, including thiamine, riboflavin, pyridoxine and niacin. Boza is widely consumed in Turkey (Yeğin and Uren, 2008; Prado *et al*, 2008) and in other countries of the Balkan Peninsula.

Kvass- Kvass is a cereal-based beverage, used mostly as a type of soft drink. Traditionally, it is produced from rye and barley malt, rye flour and stale rye bread. Kvass is a very popular beverage in the countries of the former Soviet Union, especially in Russia (Dlusskaya *et al*, 2008). Kvass is a sparkling, sweet or sour beverage with a rye bread flavour and golden-brown colour. Kvass contains carbohydrates (mainly maltose, maltotriose, glucose and fructose), proteins and amino acids, lactic and acetic acid, ethanol, minerals and vitamins. Its microbiota of fermentation consists of LAB (Lb. casei, Ln. mesenteroides) and yeasts (NCBE, 2002; Costa *et al*, 2013).

Hardaliye - Hardaliye is a fruit-based non alcoholic frmented beverage. It is made from red grape juice and crushed black mustard seeds. Hardaliye originates from Thrace, in the European part of Turkey, where it is widely consumed. The microbial population of hardaliye has been reported to be mainly composed of lactobacilli and unknown fungal species including Lb. paracasei subsp. paracasei, Lb. casei subsp. pseudoplantarum, Lb. brevis, Lb. pontis, Lb. acetotoleran, Lb. sanfransisco and Lb. vaccinostercus (Aydoğdu *et al*, 2014; Güven and Aksoy, 2009).

Kombucha - Kombucha is one of the most popular LAFB in the world. Black tea and white sugar are used for its production, the drink was originally popular in China (Cvetković *et al*, 2008). The microbiota of kombucha's fermentation has been examined by many research groups, which concluded that both LAB and yeasts are present during the fermentation (Tamang and Kailasapathy, 2010). Traditionally, it was a homemade drink and the preservation and supply of the symbiotic colony of bacteria and yeast was included in the process, but nowadays it is also commercially available (Nguyen *et al*, 2015).

Amazake - Amazake is a sweet fermented rice beverage that is the non-alcoholic precursor to sake, produced in Japan. Steamed rice is mixed with rice-koji (Aspergillusmycelia and rice) and water, and is heated to 55 degree celcius for 15 to18 h. Enzymes breakdown the rice and form glucose content of approximately 20%. Amazake is highly nutritious and is consumed for its purported health benefits (Yamamoto *et al*, 2011).

In Asia the preparation of fermented foods is a widespread tradition. India, being a huge country has been the home of innumerable religious order of human population and diversity in climatic conditions has resulted in a large number of fermented foods. Most of these remain majorly secretive in their preparation being passed on from generation to generation and tend to be regionalized with many of them being made only on the home scale by using back slopping (Chilton *et al*, 2015). Fermented beverages have heterogeneousness of traditions and cultural preferences found in the different geographical areas, where they are produced. They have been consumed since ancient times due to their prolonged shelf life, reduced volume, shorter cooking times and superior nutritive value as compared to the non-fermented ingredients. There are different fermented products in India such as Dahi, lassi, Kadhi, Soor, ghanti, jann, etc. (Savitri and Bhalla, 2007).

In western countries, numerous varieties of beverages are prepared by means of barley malt, as a source of starch and saccharifying agent. In contrast, in the preparation of Indian traditional beverages such as ragi, rice and barley are used as a rich source of starch and saccharifying enzyme. Many varieties of alcoholic beverages are manufactured and consumed by ethnic people of India. Traditionally different fermented beverage are been produced at different part of India, depending upon the geographical condition, climatic condition and the raw food material chiefly available in that locality of area (Rawat *et al*, 2018). Fermented beverages like Soor, ghanti, jann, daru are popular drink of himalyan belt or himalyan region, but there is a variation in the raw material used, rice, barley or finger millet is used as raw materials for the preparation of soor, which contains 35-40 % alcohol. The people of Himalayan region such as Ladakh call Ghanti as chang.

Dahi- Traditionally, dahi is a naturally fermented milk product obtained from boiled cow or buffalo milk and soured using lactic cultures as Lactococcus lactis spp. lactis, Lactococcus lactis spp. cremoris, and L. diacetylactis it used separately or in combination. It is used in daily diet as a potential source of B-complex vitamins, folic acid, and riboflavin (Sharma and Lal, 1997, Sarkar *et al*., 2015). Dahi is rich in lactic acid bacteria and demonstrates the probiotic effect, which helps in intestinal health as helps in controlling diarrhea in children (Agarwal, 2002).

Raab/ Raabdi- Raab is a famous dairy product, prepared in the north-west part of India. It is a lactic acid-fermented milk product with pearl millet. Pearl millet has a lower glycemic index as compared to other cereals, which is helpful in managing noninsulin dependent diabetes mellitus, where primary cause is associated with an interruption in carbohydrate metabolism (Sarkar *et al*., 2015).

Lassi- Lassi is a traditional fermented milk beverage consumed in summer season for refreshment and is prepared from dahi. It is prepared by blending dahi with water, sugar, salt, and spices such as cumin seeds and coriander leaves. The presence of spices decreases thirst. Lassi is a probiotic product due to the presence of L. acidophilus and S. thermophilus as active cultures (Patidar and Prajapati, 1998; Sarkar *et al*., 2015)

Buttermilk- Buttermilk is the liquid that is left over when butter is churned out of cream or dahi, consumed with or without added salt and spices. It has less fat content and fewer calories compared with regular milk or dahi. Buttermilk is as rich a source of calcium and protein as like milk. In some states of India, it is also known as Chaach. It is easier to digest and, with the presence of live cultures, helps in intestinal health. Buttermilk is consumed with meals and is advised in the Ayurveda (Pushpangadan *et al*., 2012)

Kadi, churpa/churpi and nudu- In Himachal Pradesh, traditional milk based products are prepared from the milk of several species of indigenous cattle, buffalo, sheep, goats, and Churu (hybrid of cow and yak) (Savitri and Bhalla, 2007). The predominant microflora is *Lactobacillus plantarum, L. curvatus, L.fermentum, L.paracasei subsp. pseudoplantarum* and *Leuconostoc mesenteroides* (Tamang *et al*, 2005; Tamang *et al*, 2009).

Ambali- It is a finger millet-based fermented semi-liquid product of south India. It prepared by mixing of finger millet flour with water to make thick batter and followed by cooking and fermentation. The fermentation process decreases the leucine to lysine ratio (from 3.6 to 4.1) and increases in concentration of thiamine (Vit B1), riboflavin (Vit B3), and tryptophan and bioavailability of minerals. The dominant microorganisms responsible for the fermentation of finger millet are *Leuconostoc mesenteroides*, *Lactobacillus fermentum* and *Streptococcus faecalis* (Ramakrishnan, 1980).

Jann- It is a traditional local drink of Uttaranchal, which is prepared from rice, koni, wheat, jau (Şanlier *et al*, 2017). It contains very low concentration of alcohol. Daru is a distilled liquor containing ethyl alcohol at a much higher concentration prepared from rice, jaggery, koni, chuwa, oowa (Sarkar *et al*, 2015). On the other hand lungi, tchang, Jhar, Zutho, Jnard, Atingba, apong, judima and baathi janr are popular product of north eastern region specifically from Nagaland, Tripura, Sikkim, Meghalaya (Kumar *et al*, 2013).

Apong- Apong is prepared by a combination of ash of paddy husk and straws, cooked glutinous rice and traditional starter and moved into an earthen pot, and are allowed to ferment for 20 days at 30 to 35°C. Many studies have found apong to be helpful in preventing kidney stones formation (Ray *et al*, 2016).

Judima- It is prepared through mixing the starter powder humao with air dried boiled rice (1:100) and fermented at room temperature. The drink is very useful against infection and is anti-inflammatory, antiallergic, antioxidant, antibacterial, antifungal, antispasmodic, hepatoprotective, hypolipidemic, neuroprotective, hypotensive, antiaging, and antidiabetic (Ray *et al*, 2016).

2.5 Probiotics and prebiotics in foods

Probiotics are defined as "live micro-organisms that confer a health benefit on the host when consumed in adequate amounts (WHO/FAO, 2001)." Probiotic bacteria should be safe for consumption, reach the intestines alive in large numbers, and impart specific health benefits to the host. These bacteria should maintain the balance of the intestinal flora by altering favorably the gut environment in such a manner that the growths of friendly beneficial bacteria are promoted and harmful disease-causing organisms are inhibited. Some of the commonly used probiotic bacteria include Lactobacillus, Bifidobacteria, and the yeast Saccharomyces boulardii. Apart from their use as drugs, they are most commonly used in the form of probiotic dairy products and probiotic fortified foods (Fijan, 2014).

Probiotics are microorganisms that transfers a tiny number of congenital health benefits to the host and have numerous applications in food and medicines. Application of probiotics in a wide range of food and drink delivery format, and have multiple health extension platform enhancements that are sold. Provide additional benefits beyond the promise of taste and convenience more and more new products (Rijkers *et al*, 2011).

The prebiotic word was first introduced by Gibson and Roberfroid in 1995 and is defined as "a non digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson and Roberfroid, 1995). In common terms, bacterial species that are believed to be beneficial for health and well-being of humans use prebiotics as food. The fermentation features selecting a substance as prebiotic should include nutraceutical effects that extend beyond those of daily nutrition. The greater number of these effects have dealt with optimized colonic function and metabolism, such as increase in the expression or change in the composition of short-chain fatty acids, increased faecal weight, a mild decrease in luminal colon pH, a decrease in nitrogenous end-products and reductive enzymes, an increased expression of the binding proteins or active carriers associated with mineral absorption, and immune system regulation (Bournet *et al*, 2002; Forchielli and Walker, 2005). On the basis of fermentability alone many substances and foods are being considered prebiotic (Blaut, 2002; Winter, 2006). Various whole foods are also being considered as prebiotics because of having different types of fibers, some of which are fermentable (Dixon, 2006; Charalampopoulos, 2002).

Fermentable fibers in particular to other fibers are crucial for good health. But prebiotics are specified ingredients which are aimed to affect the specific bacteria, end- products of their fermentation, and possible health benefits on the host. So, prebiotics are typically oligosaccharides or more complex saccharides that are selectively used by commensal bacteria, including species considered to be beneficial for the human host. Prebiotics must resist host digestion, absorption, and adsorption before fermentation by one species of the resident microbiota (Gibson, 2008). The most common prebiotics are inulin, its derivatives fructooligosaccharides (FOSs) and galactooligosaccharides (GOSs), however, other complex saccharides and fibers have been recently considered as prebiotics. The most commonly used prebiotics to develop the foodstuffs, including baby foods, are fructans (inulin and fructo-oligosaccharides, FOS) and galacto-oligosaccharides (GOS) (Kolida and Gibson, 2007; Fanaro *et al*, 2005). They are believed as GRAS and their health-benefiting effects have been widely studied.

Prebiotics may exhibit the following properties: · Maintenance of intestinal flora and stimulation of intestinal transit (Arabbi *et al*, 2001); · Change in colonic microflora, contributing to normal stool consistency, preventing diarrhea and constipation (Bosscher *et al*, 2006; Ouwehand *et al*, 2005; Macfarlane *et al* 2006; ; · Elimination of excess substances such as glucose and cholesterol, favoring only the absorption of substances needed (Kaur and Gupta, 2002); Stimulation of the growth of bifidobacteria (Losada and Olleros, 2002); · Stimulation of the absorption and production of B vitamins (B1, B2, B3, B6, B9, B12) (Wang and Gibson, 2993); · Support of the immune system (Silva and Nornberg, 2003); · Contribution to the control of obesity (Manning and Gibson, 2004); and · Contribution to the decrease of the risk of osteoporosis (Kaur and Gupta, 2002).

2.5.1 Probiotic products in India and the world

Probiotic bacteria are present all over the world in a number of products, such as traditional food products, dietary supplements and medical foods. The principal suppliers for probiotic bacteria are dairy foods and dietary supplements (mostly available as capsules, powders or tablets) (Sanders, 2000). Dairy food products incorporating probiotic bacteria are carried by almost all major brands of yogurt, culture-containing fluid milks, which includes “Sweet Acidophilus Milk” along with some brands of cottage cheese. Dairy foods products appear to fit naturally with probiotics due to the traditional relation of important fermentation bacteria and also fermented milk and other dairy products. Consumers naturally associate fermented dairy products with live cultures and recognize a benefit (albeit undefined) in the presence of these cultures (Sanders, 2000)

2.5.2 Probiotics – in Indian scenario

Within the functional foods, probiotics is a quickly developing, active field. In the probiotic revolution, India should be able to play an important role being the leading manufacturer of milk and having the world’s highest cattle population. Indian probiotic sector is in an infancy stage and currently represents only a small percentage i.e. less than 1% of the total world market revenue in the probiotic markets (Probiotic Association of India 2011). However the probiotic sector is growing at a sound speed with the situation set for incredible development in the future. India is appearing to be a huge probiotic industry of the upcoming days with an annual development speed of 22.6% until 2015. The few competitors in the Indian probiotic sector are AMUL, Mother Dairy, Yakult Danone and Nestle as well as other minor players working in various regions in independant capacities. With their onset, the Indian probiotic sector revenue is estimated to reach $8 million by the year 2015. Probiotics in India usually come in two forms, milk and fermented milk products with the former occupying 62% of the market share and the latter having 38% market share (Indian consumer survey, 2010). AMUL was the initial brand to produce a knock at the National level with it’s probiotic ice cream ‘Prolife’ in February, 2007. AMUL, additionally, tasted popularity in the probiotics field with its ice cream and therefore introduced probiotic lassi. Probiotic products contribute 10% to it’s ice-cream business and 25 per cent to it’s Dahi (Indian yoghurt) business. Mother Dairy carries the largest milk (liquid/unprocessed) plants in Asia manufacturing above 25 lakh liters of milk per day. b-Activ Probiotic Dahi, b-Activ Probiotic Lassi, b- Activ Curd and Nutrifit (Strawberry and Mango flavours) are the company's probiotic products. Probiotic products are adding around 15% of the turnover of their fresh dairy products. Nestle NESVITA was India's first Dahi with Probiotics – For Healthy Digestion. Yakult Danone India Pvt Ltd (YDIPL) is a 50:50 joint venture between Japan's Yakult Honsha and The French-Danone Group and is offering Yakult, a probiotic drink made from fermented milk, Lactobacillus and some sugar. The introduction of Yakult is expected to improve the awareness and expansion of the probiotic class in India. Leading companies in the probiotic drug market in India include manufacturers like Ranbaxy (Binifit), Dr. Reddy's Laboratories, having four probiotic brands, Zydus Cadila, Unichem, JB Chem, and Glaxo SmithKline. While probiotics by means of drugs are well-known, probiotic foods are still viewed with scepticism. Popularization is increasing slowly, but it takes a long time to alter the approach of the Indian consumers.

2.5.3 Global Scenario of Probiotics

The expansion of probiotic products in the established world is quite outstanding. As per a new market research report, 'Probiotics Market' (2009-2014) published by Markets and Markets, the worldwide probiotic market is expected to be worth US $ 31.1 bn by 2015 with Europe and Asia accounting for approximately 42 and 30% of the entire turnover, respectively. Europe formed the highest market for probiotics which was nearly $13.5 billion by 2014. Asia is the second most important marketplace, expanding with an estimated CAGR of 11.2% to reach $9.0 billion by 2014. A Frost and Sullivan study estimates that the probiotic ingredient market in the US was $450 million in 2010. Probiotics, with a market of $3.23 billion is a huge section of the Japanese functional foods. The Japanese spend $126 per individual per year on functional foods which is greater than other nations including US ($67.9), Europe ($51.2) and Asia ($3.20). Based on Euromonitor International's packaged food data, in 2000, pro/prebiotic yogurt (both drinking and spoonable combined) accounted for one-quarter of worldwide yogurt business by value. A decade later, in 2010, it accounted for one-third, global worth for sales of probiotic dietary supplements nearly tripled, amounting to US$2.2 billion in 2010.

2.5.4 Use of probiotics in foods

Dairy products work as useful delivery vehicles for probiotic bacteria. Probiotic bacteria possess a prolonged history of connection with dairy products. It is due to a number of identical microorganisms which are related to fermented dairy products that they have acquired their homes in various areas on the human body, including the mouth, the gastrointestinal tract and so on. Many of these microorganisms, thus, can participate in the dual role in converting milk into distinct and wide varieties of fermented dairy products (yoghurt, cheese, kefir, etc.), and also leading to the vital role of colonizing bacteria. The increasing awareness of consumers in the direction of therapeutic products has further resulted in the incorporation of probiotic cultures into a variety of milk products (Panesar 2011). Studying the background of probiotics offers a traditional explanation for exactly why dairy products—specifically, yogurt like products—form the major segment by far of the market for probiotic products. The effect of this history with respect to consumer perception are that: 1. Fermented dairy products such as yogurt already have a record as being healthful. 2. Consumers are familiar with the fact that fermented products contain viable microorganisms. 3. Probiotics as fermentation organisms combine the positive images of both probiotics and fermentation organisms. 4. The image of yogurt like products as healthy foods facilitates the recommendation of daily consumption of probiotics. Besides, there is the essential technologic reason for the exploitation of dairy products as carriers of probiotics: many of these products have already been optimized to some degree for the survival of the fermentation organisms. Therefore, present technologies can be relatively effortlessly adapted to promise adequate survival of the added probiotic bacteria.

2.5.4.1 Fruits and probiotic product development

A fruit is the edible portion of plant. They are usually sweet, with a wide range of colour, flavor and texture. Consumption of fruits is important for human health because these foods are primary sources of some essential nutrients and contain phytochemicals that may lower the risk of chronic diseases (Kunnumakkara 2014) Wine grape pomace was incorporated as the antioxidant dietary fibre for enhancing the nutritional value and improving the storability of yogurt and salad dressing (Tseng and Zhao 2013). Passion fruit peel powder was analysed for fermentation kinetics, texture profile and bacterial viability in probiotic yoghurts (do Espírito Santo *et al*. 2012). Cheese was prepared using *Lactobacillus casei* cells immobilized on apple and pear pieces (Kourkoutas *et al*. 2006) Flavoured yoghurt was prepared using non – cultivated indigenous swazi fruits (Dlamini *et al*. 2009). Yogurt prepared using L*actococcus lactis* immobilized within pineapple and yam bean segments, and jerusalem artichoke powder (Phuapaiboon *et al*. 2013). Probiotic yogurt prepared using thai fruits (Thumrongchote, 2014), have also been used for probiotic product development.

2.5.4.2 Dairy products

Yogurt: Yogurt is a cultured dairy product that is widely consumed as a healthy and nutritious food and for its sensory properties. It is produced from milk by fermentation, one of the oldest methods practiced by human beings for the transformation of milk into products with an extended shelf life (Tamime and Robinson, 1999). Today's yogurt is one of the most popular dairy products in the world and also proven as a good carrier for probiotic microorganisms.

Lassi: Lassi is considered as one of the thirst quenching beverages. Lassi is a popular indigenous fermented milk beverage which is usually prepared by mixing dahi and water. It is consumed as a cold, refreshing therapeutic beverage in summers in India (Aneja *et al*., 2002).

Shrikhand: Shrikhand is a popular fermented dish consumed by Indians during festival and wedding occasions. Varieties of shrikhand are available in the market with different flavors and aroma by incorporating different fruit pulps and spices.

Ice cream: Ice cream is a popular dairy product worldwide which is consumed by the general population. It is also suitable as a food carrier for probiotic bacteria. Compared with fermented milks as a vehicle, ice cream supports considerably greater viability of probiotic strains during production and especially storage (Mohammadi *et al*., 2011).

2.5.5 Use of prebiotics in foods

Prebiotics are being used in the food industry as functional ingredients in beverages (fruit juices, coffee, cocoa, tea, soft drinks and alcoholic beverages), milk products (fermented milk, milk powder and ice cream), probiotic yogurts and symbiotic products (Gibson and Roberfroid, 1995; Mussatto and Mancilha, 2007). Other applications include desserts (e.g., jellies, puddings, fruit-flavored ice cream), confectionery items (e.g., sweets), biscuits, breakfast cereals, chocolates, breads and pastas, meat products (e.g., fish paste) and tofu. Prebiotics can also be used in cosmetics, pharmaceuticals and products for people with diabetes.

Both important technological characteristics and interesting nutritional properties have been shown by Prebiotics (Huebner *et al*, 2007; Huebner *et al*, 2008). Some of them are found in fruits and vegetables and some can be processed industrially from renewable materials. In foods they affect considerably by improving organoleptic characteristics, enhancing both taste and mouth feel. As functional food ingredients prebiotics must be chemically stable to processing treatments of food, such as high temperature, low pH, and Maillard reaction conditions. Therefore, if the prebiotic was degraded to its component mono- and disaccharides or chemically altered, a prebiotic would no longer provide selective stimulation of beneficial microorganisms and it was unavailable for bacterial metabolism (Bohm *et al*, 2005). A study ascertained the effect on the prebiotic activity by processing conditions of commercial prebiotics using a prebiotic activity assay. The results showed that a significant decrease in prebiotic activity was only seen when heating at low pH, with one of the fructooligosaccharides (FOS) products being the least stable. Little change in activity was caused by other conditions (Bohm *et al*, 2006; L'Homme *et al*, 2003). These results help us in selecting of prebiotics as functional food ingredients and predicting the extent to which processing affects prebiotic activity. Presently known prebiotics and prebiotic candidates are nondigestible oligosaccharides. They are acquired either by extraction from plants (e.g., chicory inulin), enzymatic hydrolysis (e.g., oligofructose from inulin) or by synthesis (by trans-glycosylation reactions) from monoor disaccharides such as sucrose (fructooligosaccharides) or lactose (trans-galactosylated oligosaccharides or galactooligosaccharides) (Moshfegh *et al*, 2009). Among these prebiotics, inulin and oligosaccharides have been most studied prebiotics and recognized as dietary fibers in most countries (Franck *et al*, 2002). Prebiotics can be used to offer a double benefit such as an improved organoleptic quality and a better-balanced nutritional composition (Nelson, 2002). As fiber ingredients the use of inulin and nondigestible oligosaccharides is simple and often leads to enhance taste and texture. Specific colonic bacteria, such as bifidobacteria and lactobacilli species readily ferment these particular forms of dietary fibre and by raising their cell population with the parallel production of short-chain fatty acids (Sghir *et al*, 1998). These acids, mainly butyrate, acetate, and propionate provide acidification of the bowel as well as metabolic energy for the host (Schaafsma *et al*, 2008). Lactose occurs wholly in the milk of mammals and one might observe from a teleological point of view that lactose ingestion provides specific health benefits beyond just being a source of energy to the suckling animal (Boehm *et al*, 2007). In fact, it is used in different foods such as in baby foods, cakes, biscuits, chocolates, sugar confectionary, and soups and sauces and the global demand has grown considerably over the previous 10 years to amount to approximately 500,000 ton per annum (Donovan *et al*, 2006). Currently, more and more prebiotics are used in different foods as an ingredient which helps in the growth of health promoting colon microorganisms particularly probiotics and offer additional health benefits. Some of the recent works done in the development of prebiotic foods are as follows:

2.5.5.1 Prebiotics in infant formula

There are number of prebiotic substances present in human milk, the most abundant of which are oligosaccharides (Brussels *et al*, 2003; Arslanoglu *et al*, 2007). Many commercially available dietary food supplements are also added with oligosaccharide prebiotics. Concerning to their addition to infant food, the European Commission's Scientific Committee on Food concluded in 2003 that the addition of oligosaccharides to infant formulas had no major worry and including the studied infant formulas (formulas adapted especially for 6- to 12-month-old infants), up to a total concentration of 0.8 g/dL in ready-to-feed formula products (Boehm *et al*, 2002). Few randomized controlled trials have been examined to see the effects of fortifying prebiotic oligosaccharides to infant foods (Moro *et al*, 2002; Schmelzle *et al*, 2003). A study showed the effect of the addition of oligosaccharides having a concentration of 1 g/dL to newly born infant formula for 1 month having 90% of GOSs and 10% of FOSs. In the oligosaccharide supplemented group the faecal bifidobacteria counts increased significantly when compared with the nonsupplemented group and the counts of bifidobacteria reached the range of a breastfed reference group. In another study, it was shown that the infants fed with the same oligosaccharide fortified formula. These infants had higher bifidobacteria counts as well as lactobacilli in their faecal matter. A multicenter trial was conducted that examined the efficiency of the fortification of prebiotics to infant foods. They stated the good overall tolerance and no undesirable effects during the study period of 12 weeks (Bettler and Euler 2006). Also a large multicenter trials have been evaluated by United States in 2004 on the safety of FOS supplemented infant formula. The study demonstrated the effect of FOS supplemented infant formula for 12 week study period and was seen that the infant growth was maintained without having any adverse effects (Scholtens *et al*, 2006). In weaning infant food formulas the fortification of prebiotics to solid foods shows to have a bifidogenic effect, as revealed by the results of recently published randomized controlled trials (Cardarelli *et al*, 2007). Infant food formulas containing either GOS or FOS are now marketed in the United States. On the other hand more information, including data from randomized controlled trials, is required before the efficacy of adding prebiotics to infant food formulas can be determined.

2.5.5.2 Prebiotics in cheese

Dairy products are considered a potential vehicle for prebiotics and probiotics and are increasingly being used to produce synbiotic foods. A research conducted to evaluate the potential of prebiotic in novel petit-suisse cheeses using an in vitro fermentation model. Prebiotics candidate (inulin, oligofructose, honey) and probiotics (*Lactobacillus acidophilus, Bifidobacterium lactis*) were combined in five petit-suisse cheese formulations and were tested in vitro with human faecal slurry. It was seen that with addition of prebiotics to a probiotic cheese (made using starter + probiotics) fastest fermentation and high lactic acid production, promoting increased growth rates of bifidobacteria and lactobacilli, were achieved. Addition of probiotics as starter culture to control cheese also resulted in high lactic acid production. In vitro conditions, cheese made from the combination of different prebiotics and probiotics shows potential functional petit-suisse cheese (Rodrigues *et al*, 2012). An investigation conducted to see the influence of prebiotic compounds (FOS and inulin), probiotic bacteria (*Lactobacillus casei-01, Bifidobacterium lactis B94*) and ripening time (0-60 days) on the free fatty acid (FFA) profile of cheese, with special prominence on the conjugated linoleic acid (CLA) content. 109-1010 cfu g-1 cheese were recorded in both probiotic and synbiotic cheeses after 60 days of ripening, in spite of harsh conditions of low pH values (4.1-5.1) and low moisture content (<30%, w/w). It was observed that the increases in total FFA and CLA during the ripening period, particularly in synbiotic cheeses having FOS and inulin (50:50) inoculated with B. lactis B94. Addition of prebiotic compounds in probiotic cheese is advantageous so far as functional CLA compounds are concerned plus an enhanced nutritional quality (Debon *et al*, 2010).

2.5.5.3 Prebiotics in fermented milk

The physicochemical composition (day 1 of storage) and rheological (days 1, 7, 14 and 21) were evaluated for the microfiltered cow's milk samples fermented with and without inulin, at temperatures of 4.0 ± 0.1ºC and 6.0 ± 0.1ºC. The total solid content of the prebiotic fermented milk increased with the addition of inulin, also increased its acidity. The Power Law and Mizhari and Berk models described the flow of the fermented milks as shear thinning and non-Newtonian fluid behaviour. The apparent viscosity of the product added with inulin increased with storage time. The prebiotic fermented milk showed greater thixotropy and the hysteresis got lowered with an increase in the temperature (Debon *et al* 2012). Debon *et al*. used permeate obtained from the best microfiltration process for the preparation of fermented milks, with and without of inulin (5 g 100 g-1), stored at 5 ± 1ºC for 28 days. It has been seen that the storage period and addition of inulin increased the total solids and carbohydrate contents, the caloric value and the acidity but decreased the pH. Lower syneresis index resulted with the addition of inulin in a fermented product also with greater firmness and cohesiveness. The inulin addition in a product resulted with a greater tendency toward a greenish coloration (Cruz *et al*, 2013). Cruz *et al*. evaluated the effect of addition on physicochemical, rheological and microbiological characteristics of non-flavoured yogurt. There was no influence on the pH, proteolysis or the viability of Streptococcus thermophilus or *Lactobacillus bulgaricus* during 28 days of refrigerated storage with the addition of oligofructose. Yogurt added with oligofructose was characterized as a weak gel, showing thixotropic and pseudoplastic behaviour as per the rheological measurements. As per the survival analysis 25% of the consumers rejected the yogurt added with different levels of oligofructose, and the level of oligofructose that can be added to the yogurt was shown to be 2.58% wt. V-1 (Guergoletto *et al*, 2010).

2.5.5.4 Prebiotics in dairy fruit beverages

Guergoletto *et al*. evaluated the survival of *Lactobacillus casei* (LC1) when adhered to dehydrated prebiotic fibers. After vacuum drying in oat bran with 9% β-glucan and green banana flour, viability of LC-1 was 79% and 76%, respectively. Images revealed by scanning electron microscopy showed no morphological changes in the cells adhered to these fibers and the cell protectant trehalose addition had a positive and significant effect on the survival of LC-1. Greater viability of LC-1 has been seen when adhered to the oat bran (7.1 log CFU g−1) than in the free form (2.4 log CFU g−1). Sensory evaluation showed that the probiotic oat bran added to a dairy fruit beverage was well accepted by consumers (Damen *et al*, 2012).

2.5.5.5 Prebiotics in cereals

Cereals and millets have been an essential part of the human diet since the beginning of agriculture and are most economic source of energy (Weaver *et al*, 1981). These are nutritionally rich and occupy an important place in the diet of people in many regions of the world. Rice and barley are commonly consumed cereals in Indian households. Barley is also good for digestive and nervous system as it contains an important amino acid L tryptophan and is helpful for sleeplessness, depression, anxiety and premenstrual syndrome. Barley as a functional ingredient has many health benefits including preventing cardiovascular diseases and cancer, lowers blood pressure, controls cholesterol level and lowers the incidents of heart diseases, reduce the rate of fat absorption, delays gastric emptying and improve gastrointestinal health (Achi *et al*., 2015). Clinical and epidemiological studies reported that β-glucan from oat and barley based products control cardio vascular disease in human beings (Beck *et al*., 2010)

Several studies have publicized the prebiotic activity of cereal derived arabinoxylan oligosaccharides (AXOS). A study investigated the in situ AXOS production during bread making process. Breads fortified with rye or wheat bran treated with HjXynA yielded high-quality breads with AXOS levels above 2.0% with an average degree of polymerization of 26 and 19, respectively (Morais *et al*, 2014). Due to wide occurrence of celiac disease and wheat allergy has led to an increasing demand for gluten-free foods that present an appropriate sensory acceptance. A consumer test was performed with 65 celiac people. Additionally, 15 trained assessors were used in the sensory profiling by using quantitative descriptive analysis (QDA). By using QDA, the samples differed significantly with respect to all parameters, and partial least squares (PLS) regression was used to recognize the factors of liking of gluten-free breads. The results show that the softness, traditional bread aroma, sweetness and crumb color are the most desired sensory properties of such products which can be considered as drivers of liking of prebiotic gluten free breads (Tuohy *et al*, 2001). In a clinical study partially hydrolysed guar gum (PHGG) and fructooligosaccharides (FOS) were incorporated in biscuit and was assessed in human volunteers. The study showed consumption of biscuits added with FOS and PHGG gave a significant increase in faecal bifidobacterial numbers (P=2.15×10-5). Bifidobacteria increased from 9.18 log10 cells/g faeces to 9.59 log10 cells/g faeces after the experimental treatment. There was small change in the total numbers of bacteria, Bacteroides spp., Clostridium spp. or *Lactobacillus-Enterococcus* spp. present in faecal samples collected over the course of the trial. No changes were observed in bacterial populations on eating of the placebo biscuits (Mantzouridou *et al* 2013).

2.5.5.6 Prebiotics in salad dressing

A study conducted by Mantzouridou *et al*. to develop a low-inoil salad dressing emulsion, based on oat flour, that has both health promoting effect as well as provides growth to probiotic *Lactobacillus paracasei subsp. paracasei* DC412 with the functional properties also e.g. emulsion stabilization and thickening ability. Probiotic viability, following emulsion storage and enzymatic treatment, was persistent in oat flour presence at the levels near to those found for inulin. The dressing emulsion was further investigated by applying response surface methodology and the optimum formulation (g/100 g) was as: oil (20), xanthan (0.475) and oat flour (3.0); viable cell counts of emulsions reached levels as high as 108 cfu/g after the refrigerated storage period of 7.5 weeks and treatment with simulated gastric and intestinal juices. In addition to enhancing cell viability, appreciable improvement of the emulsion rheological properties and physical stability of salad dressing occur by oat flour incorporation (Huezo *et al*, 2007).

2.5.5.7 Prebiotics in custard and ice creams

Rheological and sensory properties were studied for low-fat custards added with (7.5 g/100 g) of long-chain inulin (TEX) and short-chain inulin (CLR) combined in different proportions (25:75, 50:50, and 75:25). Low-fat samples added with inulin blends did not cope to fully imitate the rheological behaviour of full-fat custard. However, inulin blends in the ratio of 50:50 of TEX: CLR added to the low-fat samples perceived to have the same creaminess and thickness as the full-fat sample. When this blend was used in combined with carrageenan gave thicker, creamier custard, which was preferred over the full-fat custard (Tarrega *et al*, 2010). A seven-day period of refrigerated storage was studied for the changes in rheological properties and microstructure of inulin-enriched custard desserts.

2.6 Use of probiotics and prebiotics in cereal based beverages

2.6.1 Probiotics in cereal based beverages

Lactose intolerance, milk allergy and the cholesterol content are major downsides encountered by dairy-based probiotic products, and with an increase in veganism there is also a demand for dairy-free vegetarian probiotic products. Also the addition of fruit and vegetable juices with probiotic bacteria has been studied as these are already positioned as healthy food products and are valued for their nutrient content such as minerals, dietary fibre, antioxidants and vitamins (Heenan *et al*. 2004; Shori 2016). However, sensorial studies performed in these products have shown that they can develop off-flavours such as medicinal and savoury, which could negatively affect their acceptance within the market of probiotic foods (Luckow *et al*. 2005, 2006). Cereals present the most potential as an alternative for the creation of nondairy probiotic foods. Their recognized health-promoting effects can be exploited leading to the development of cereal-based probiotic products. As shown in Table 2.1, cereals can be applied as nutrient sources that besides promoting several beneficial physiological effects can also stimulate the growth of lactobacilli and bifidobacteria (Rathore *et al*. 2012; Herrera-Ponce *et al*. 2014).

Table 2.1 List of novel probiotic cereal beverages

Sources	Feature	Probiotics	References
Malt, wheat, barley and oat	Cereals are able to support bacterial growth and increase their acid and bile tolerance	*Lactobacillus fermentum, Lactobacillus reuteri, Lactobacillus acidophilus, Lactobacillus plantarum*, and *Bifidobacterium sp*	Charalampopoulos *et al*. (2003), Patel *et al*. (2004), Rozada Sanchez *et al*. (2008), Michida *et al*. (2006)
Oat, malt	Fermentations with different fractions of cereal grains exhibit higher probiotic levels than those obtained with the use of the whole grain	*L. reuteri, L. acidophilus*, and *L. plantarum*	Kedia *et al*. (2007), Kedia *et al*. (2008)
Barley, malt	Mixed-culture fermentations in mixed and single cereal substrates produced similar values of cell units, but the production of organic acids was significantly different. Therefore, organoleptic properties are unique of each fermented cereal beverage.	*L. acidophilus*, and *L. plantarum*	Rathore *et al*. (2012)
Oat, germinated oat, malted oat	Different inoculum levels did not exhibit a significant effect on probiotic lactobacilli growth. It was observed that protein supplemented simple, germinated and malted oat improved the cell viability of the probiotic strains.	*Lactobacillus rhamnosus, Lactobacillus casei*, and *L. acidophilus*	Herrera-Ponce *et al*. (2014)

Prebiotics in cereal based beverages

Some of the health benefits driven by the consumption of cereal prebiotics in human diet are the reduction of diseases such as diabetes, hypertension, coronary heart disease, obesity and gastrointestinal disorders such as colorectal cancer (Berger *et al*. 2014). The most common prebiotics used are carbohydrates of low digestibility namely xylooligosaccharides (XOS), pectic-oligosaccharides, mannooligosaccharides, transgalactosylated-oligosaccharides, chitooligosaccharides, arabinoxylanoligosaccharides (AXOS) and galactooligosaccharides (GOS) (Table 2.2) (Aachary *et al*. 2011; Damen *et al*. 2011; Duncan and Flint 2013). The study of cereals and their health-promoting effects following consumption has given a special attention to the evaluation of whole grain fibre due to its prebiotic effects (Alminger and Eklund-Jonsson 2008). The consumption of whole grains confers the health benefits of fibre and provides other micronutrients such as unsaturated fatty acids, and lignans. Fibre is present in the three main components of the grain (bran, germ and endosperm); however, the chemical composition and bioactive properties of this might vary. Cellulose is a fibre that has been reported to pose little effect on lowering blood glucose and lipids; thus, it is usually employed as a placebo in clinical trials. Another fraction of polysaccharide fibres is hemicelluloses linked with plant cell wall components such as phenolic compounds, lignins, proteins and cellulose. The most important hemicelluloses are b-glucans and AXOS due to their health benefits followed by minor hemicelluloses such as arabinogalactans, xyloglucans and gluco- and galacto-mannans. Other important grain fibres are resistant starch and fructans that have been extensively linked to multiple health benefits (Bernstein *et al*. 2013). Prebiotics are selectively fermented by colonic bacteria that belong to several species which can be classified in three major groups: (i) Bifidobacterium and Lactobacillus, (ii) pathogenic bacteria like clostridia and (iii) other bacteria such as Bacteroides that can exert beneficial or nonbeneficial effects. Among these types of fermentations, the saccharolytic is preferred due to the production of health-recognized metabolites named short-chain fatty acids (SCFA) such as propionate, butyrate and acetate (Miremadi and Shah 2012).

Table 2.2 Cereal prebiotics for the development of healthy beverages

Origin	Component	Health effects	References
Oat and barley	Whole grain fibre	Lowering of the postprandial blood glucose and insulin responses	Alminger and Eklund-Jonsson (2008)
Oat	Whole grain	Lower the threat of cardiometabolic disorders and lower blood glucose, which makes it attractive for designing lowglycaemic load diets	Connolly *et al*. (2012)
Wheat	Arabinoxylan (AX) and AX oligosaccharides	Improved production of fermentation health metabolites SCFA	Damen *et al*. (2011)
Corn cob	Xylooligosaccharides (XOS)	Lower cholesterol levels, antimicrobial, anti-inflammatory, antiallergy and improvement of mineral absorption	Aachary *et al*. (2011)
Barley	b-glucans	Regulate food intake and can reduce 24 h energy intake	Alminger and Eklund-Jonsson (2008), Xu *et al*. (2016), Zhu *et al*. (2016)
Oat	b-glucans	Delay gastric emptying time and intensify gastrointestinal transit time, which are related with reduction of blood glucose level, and a hypocholesterolemic effect by reducing total and LDL cholesterol	Sethi *et al*, 2016
Wheat	Fructans	Selectively fermented by the colon microbiota and exert changes in the gastrointestinal microbiota conferring health benefits	Verspreet *et al*. (2015)

2.7 Fructooligosaccharide (FOS) as a prebiotic

FOS is a common name for fructose oligomers, and these are usually understood as inulin-type oligosaccharides. They constitute a series of homologous oligosaccharides derived from sucrose usually represented by the formula GFn, which are mainly composed of 1-kestose (GF2), nystose (GF3), and 1F -β- fructofuranosyl nystose (GF4), in which two, three, and four fructosyl units are bound at the β-2,1 position of glucose, respectively.

Several studies have demonstrated the functional properties of fructooligosaccharides (FOS), such as the reduction of cholesterol levels and blood glucose levels, lowering of blood pressure, better absorption of calcium and magnesium and to inhibit production of the reductase enzymes that can contribute to cancer (Pereira and Gibson, 2002; Coundray *et al*, 2003). FOS are not digested by the human gastrointestinal tract, and when they reach the colon, they beneficially stimulate the growth and strengthening of specific bacteria in the intestine (Gibson and Roberfroid, 1995). The bifidobacteria secrete ßfructosidase, which would be the enzyme responsible for FOS hydrolysis (Roberfroid, 1993). The average counts of bifidobacteria increased, whereas there were significant reductions in Bacteroides, Fusobacterium and Clostridium sp.

Requirement for FOS

Flamm *et al*., (Flamm *et al*, 2001) have evaluated the caloric value of FOS and found that the energy yield for the host would be in the range of 1.5 kcal/g to 2.0 kcal/g. By using another method based on lipogenesis balance, Roberfroid (Roberfroid, 1993) stated that the caloric value of FOS is around 1.0 kcal/g to 1.5 kcal/g. In Holland, it is estimated that the consumption of FOS is 2 g to 12 g per day. In Japan, the estimate is between 13.7 mg/kg of body weight per day. However, for the approval of FOS, the Japanese law established the amount of 0.8 g/kg of body weight per day as an acceptable daily intake (Passos and Park, 2003; Tuohy *et al*, 2005). The average per capita daily consumption of FOS is 2 - 4 g for North Americans and 2 - 12 g for Europeans (Gibson *et al*, 1994). In Brazil, there are no relevant data regarding the amount consumed or the dietary recommendations. The law considers FOS as ingredients of products, not additives. FOS are considered as dietary fiber, and in the United States, they have a GRAS status (Generally Recognized As Safe). Ingestion may cause flatulence, especially in individuals who have lactose intolerance, but the severity of this symptom is associated with the amount of FOS consumed: the higher the quantity, the greater the symptom (Hauly and Moscatto, 2002). The intake of 20 g to 30 g per day can promote severe discomfort in an individual, and thus, the optimal intake level is 10 g per day. For the promotion of colon floral balance, the amount of FOS needed has been determined to be 2 g to 2.5 g per day (Spiegel *et al*, 1994). The minimum dose of FOS for the induction of diarrhea is 44 g for men and 49 g for women (Costa and Waitzberg, 1997; Lima, 2003). For enteral nutrition, several clinical studies suggest the amount of 5 - 10 g/day for the maintenance of normal flora and from 12.5 g/day to 20.0 g/day for bifidobacteria recovery (Tokunaga *et al*, 1989). In vitro and in vivo studies have suggested the lack of genotoxicity and mutagenicity of FOS. Evaluations conducted in rats showed no adverse effects with quantities lower than 2.17 g/kg/day (Costa and Waitzberg, 1997; De Leenheer, 1996).

2.8 FOS - its types and technological function

There are three categories of FOS, each of which is structurally distinct: inulin, has a polymerization degree of 2 to about 60 monomers of fructose, with an average of 12 units (Babu *et al*, 2012): oligofrumose is produced by the enzymatic hydrolysis of inulin and is defined as a fraction of oligosaccharides with degree of polymerization lower than 20, although commercial products tend to have a mean value of 9; these FOS are produced by the enzymatic hydrolysis of inulin and consists of frucrosyl chains of different lengths, with glucose and fructose terminals. Finally, scFOS (short chain fructooligosaccharides) are specifically defined as mixed chains of fructosyl with a glucose terminal unit; they have a maximum of 5 units and are derived from sugar through natural fermentation processes, producing 1-kestose (CF 2), nistose (CF 3) and 1-fructosyl - nistose (GF 4) in which the fructosyl units (F) are linked at ß-(2-1) position of sucrose (Fig2.2).

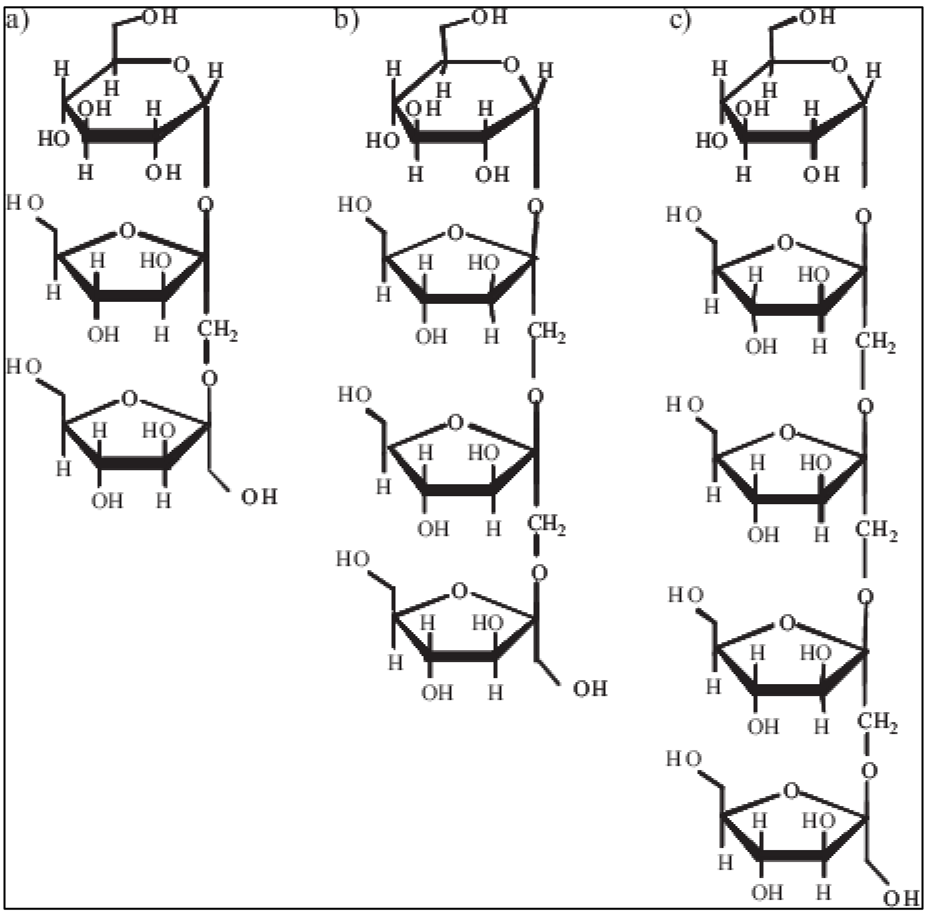

Fig 2.2 Chemical structure of relevant fructooligosaccharides:a) 1-kestose (GF2), b) nystose (GF3), c) 1-b-fructofuranosyl nyst-ose (GF4).

Fructooligosaccharides (FOS) belong to the group of oligosaccharides and are isolated from plants. In the extraction commercialization process, FOS can be obtained from inulin by means of the transfructosylation enzymatic reaction in sucrose residues by the action of the ß-fructofuranosidase enzyme, with the DP of these products varying between 1 and 7 fructosyl units (Borges, 1997)

Sources

FOS are available in some foods such as bananas, garlic, onion, tomato, wheat, asparagus, artichoke, leek, honey, rye, brown sugar, barley, triticale, beer, lettuce, chicory, burdock, beetroot, apples, bulbs like red lilies, yacon and oats, with onion being the food with the highest levels of FOS (Table 2.3).

Table 2.3 Amount of FOS (%) per 100gm of raw in some natural foods

Food	Percentage
Chicory root	22.9
Jerusalem artichoke	13.5
Dandelion greens	10.8
Garlic	5
Leek	5.2
Asparagus	2.5
Banana	0.5

2.10 Development and commercialization of innovative health beverages

Demand coming from millennial generation, rising health care & wellness awareness and busy lifestyles have led to the growing demand for convenience beverages. These factors along with the idea of “healthiness-on-the-go” and innovations in the drink flavors have led to surge in demand for health associated functional drinks and beverages which are also the alternatives to the traditional supplements. The rising health awareness of functional beverages are leading to the opportunity of manufacturing condition specific product offerings which will clearly stand out in the market.

The global functional beverage market is a growing sector of the food industry as modern health-conscious consumers show an increasing desire for foods that can improve well-being and reduce the risk of disease. Fermented milks, especially yoghurt-style products, are the most popular functional beverages with kefir in Western Europe and North America and Denmark being good examples. Dairy-based produce account for approximately 43% of the functional beverage market, and is mainly comprised of fermented products (Ozer & Kirmaci, 2010).

Energy drinks, sports drinks & nutraceutical drinks are the major segments of the global functional beverages market. The energy drink market is forecasted to be the fastest growing segment due to the steadily growing demand from health conscious consumers. Functional beverages are the emerging class of natural products which helps in reducing the risk of chronic disease beyond basic nutritional functions. The functional beverages market is one of the fastest emerging markets due to its minerals and herbs content in the drinks. It’s also used to keep blood sugar levels under control leading to increased demand.

Consumers are now looking for variations in daily drinks which include hydration with performance & even preventive measures taken for specific health conditions. The major ingredients used are vitamins, amino acids and omega-3 fatty acids. Amino acids are used to slow fatigue and vitamins are added to boost the metabolism and generate energy in functional beverages. Omega-3 is widely used and its market is forecasted to grow as it controls inflammation. Prebiotics and probiotic**s** are another ingredients widely used in these drinks to ensure proper functioning of digestive system.