REVIEW OF LITERATURE
2. REVIEW OF LITERATURE

The beneficial effects of fermented milks had its science based at the beginning of the twentieth century when Eli Metchnikoff proposed a theory about longevity of the Balkans based on daily intake of milk fermented by the LAB (González 1997). Metchnikoff (1908) believed that the metabolic activity of lactic acid bacteria inhibit intestinal bacteria in the same way that inhibits the putrefaction of foods (Adams and Moss, 1997). His publications, "The prolongation of life" and "The bacillus of long life" can be considered the birth of probiotic foods (González, 1997).

2.1. FAO & WHO on Probiotics:

An expert panel commissioned by FAO and WHO defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host (Sekine et al., 1985). The term probiotic was derived from a Greek word, meaning "for life". The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have stated that there is adequate scientific evidence to indicate that there is potential for probiotic
foods to provide health benefits and that the specific strains are safe for human use (Hilton et al., 1996).

2.2. Probiotic research: An emerging area

Probiotics represents an expanding research area. Medline search of the term probiotics illustrates the significant increase in research undertaken in this area during the last 5 years: over 5,000 publications cited, compared to 85 for the previous 25 years. In addition, although clinical evidence of the tangible benefits of probiotics is mounting, this does not yet reflect the commercial front of probiotic organisms because of the lack of following aspects:

- Well-defined and well-characterized strains.
- Randomized, double-blind, placebo-controlled studies.
- Result confirmed by different groups.
- Publication of results in peer-reviewed journals.

The probiotics are microbial supplements that positively influence the body and increase significantly the nutritional and therapeutic value of foods through
the intestinal microbial balance and physiological function of human intestinal tract (Goldin and Gorbach, 1980). Similarly, probiotic foods are defined as foods containing microorganisms that have beneficial effect on intestinal microflora and physiological functions of the human intestinal tract. Consumption of by blocking the receptors of pathogens by neutralizing the effects of enterotoxins and promoting the development of organisms resistant to pathogens, particularly against *Escherichia coli* (Lee *et al.*, 1999). Furthermore, they have ability to improve lactose digestion in persons classified as lactose-intolerant, metabolize some types of drugs to reduce cholesterol levels and risk of colon cancer (Gilliland, 1989).

2.3. **Probiotics as dietary adjuncts:**

Consumers are showing greater interest in their food as a means to maintain or improve their health. Modern lifestyles leave less time to prepare and eat food and this contributes to an unhealthy diet. Consumption of processed foods is associated with decreased numbers of beneficial gut bacteria. Other factors known to decrease survival of beneficial bacteria in the gut include stress, intake of antibiotics and consumption of
red meat and alcohol. Diminished beneficial bacteria allow the growth of undesirable bacteria in the gastrointestinal tract as well as reducing the amount of nutrients produced by the beneficial bacteria.

There has been an increasing trend in the manufacture and marketing of functional foods that affect functions of the body in a targeted manner so as to bring about positive effects on physiology and nutrition. The National Center for Complementary and Alternative Medicine (NCCAM), National Institutes of Health (NIH) interprets "functional foods" as "components of the usual diet that may have biologically active components (e.g. polyphenols, phytoestrogens, fish oils, carotenoids) that may provide health benefits beyond basic nutrition" (Springer Berlin/Heidelberg, 2004).

On the other hand growing concern over population and environmental control has compelled the dairy industry to stop dumping whey into streams and municipal sewage systems. At the same time recent investigations have established the potential nutritional value of the whey solids beyond dispute. In light of the
global food shortage, the most logical use would be to return whey to the human food chain in a palatable form. Attempts to utilize the nutritive components of whey more completely in human food formulations with minimum of energy, material, labour and processing costs are amongst the deciding factors in selecting the ways for whey utilization.

In recent years there has been much interest in low calorie food products and probiotics. Incorporation of *L. acidophilus* and *Bifidobacterium* in fermented beverages can result in a milk product with excellent therapeutic value and effective calorie reduction. Few attempts have been made to utilize whey in the formulation of ready-to-serve (RTS) beverages mainly through enrichment with fruits and vegetable juices, organic acids, protein concentrates, special flavours and colours etc. Whey beverages are beneficial to health and nutrition and their preparation hold potential for greater economic returns. Hence, a variety of whey beverages consisting of plain, carbonated, alcoholic etc., have been successfully developed and marketed all over the world, utilizing the whey solids. Keeping the
objectives of the study in mind, the literature pertaining to various aspects of probiotic whey beverages enriched with tropical fruit juices is reviewed and presented hereunder:

2.4. Types of whey in making probiotic beverages:

Whey, essentially milk depleted of casein and fat, is produced during manufacture cheese, casein, chhana, Paneer and other coagulated dairy products. It constitutes about half of the total solids of milk. Its physico-chemical characteristics vary according to the type of product from which it is derived (Kosikowski, 1979; Bhattacharjee, 1993; Sachdeva et al., 1998). Generally, whey is of two types, depending on processing sequence resulting in casein removal from fluid milk.

i). **Sweet whey**: Resulting from rennet coagulated casein (pH>6)

ii). **Acid whey**: Resulting from the process using organic or mineral acid to coagulate the casein (pH = 4-5)
2.4.1. Composition of whey:

According to its average composition, whey is approximately 93% water and contains about 50% of total solids present in the milk of which lactose is the main constituent. Whey proteins constitute less than 1% of dry matter (Beucler et al., 2005).

**TABLE 2.1: COMPOSITION (G/L) OF WHEY**

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Sweet whey</th>
<th>Acid whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids</td>
<td>63-70</td>
<td>63-70</td>
</tr>
<tr>
<td>Lactose</td>
<td>46-52</td>
<td>44-46</td>
</tr>
<tr>
<td>Protein</td>
<td>6-10</td>
<td>6-8</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.4-0.6</td>
<td>1.2-1.6</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1.3</td>
<td>2.0-4.5</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.0</td>
<td>6.4</td>
</tr>
<tr>
<td>Chloride</td>
<td>1.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Jelen (2002)

Minerals and milk fat are also present but in less amounts. However, whey composition is very variable and significantly depends on the technology of whey production. Most compositional differences are in contents of calcium, phosphates, lactic acid and lactate which are present in much higher amounts in acid whey.
The composition of whey proteins in these two types of whey is also not identical. The rennet whey contains a well-defined part of k-casein, the casein macropeptide as a result of action of chymosin. Whey proteins include several thermosensitive fractions like β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins and thermostable fraction of protease peptones. Due to the high content of essential amino acids (notably lysine, cysteine and methionine) and cystin, nutritionally whey proteins are one of the most valuable proteins.

Due to such amino acid composition whey proteins have much higher biological value (but also other parameters that determine nutritional value) in comparison with casein or other proteins of animal origin, including egg proteins which have been considered for a long time as referent proteins. Protein utilization in human beings is tightly related to cystine:methionine ratio which is about 10 times higher in whey proteins than in casein. Therefore, it is not surprising that thermally denaturated lactalbumins are being almost totally (100%) absorbed in the digestive system while this ratio is significantly lower (about 75%)
regarding casein absorption. Whey protein content is similar in sweet and acid whey. Nevertheless, it is important to mention free amino acid content which varies a lot and is mostly dependent on the level of casein hydrolysis in cheese making process. Thereby, free amino acid content is about 4 times higher in sweet whey and about 10 times higher in acid whey than in milk (Tratnik, 1998).

Recommended daily intakes of most essential amino acids can be satisfied by consumption of 1.5 liters of whey or 0.5 liters of milk (Popović-Vranješ and Vujičić, 1997).

**TABLE 2.2: AMINO ACID CONTENT (MG/L) OF WHEY**

<table>
<thead>
<tr>
<th>Whey</th>
<th>Free amino acids</th>
<th>Amino acids in proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Essential</td>
<td>Total Essential</td>
</tr>
<tr>
<td>Sweet whey</td>
<td>132.7 51.0</td>
<td>6.490 3.326</td>
</tr>
<tr>
<td>Acid whey</td>
<td>450.0 356.0</td>
<td>5.590 2.849</td>
</tr>
</tbody>
</table>

(Tratnik, 1998)

Besides, whey proteins have excellent functional properties like good solubility, good viscosity, good emulsifying and gelation abilities. Therefore whey protein concentrates are largely used in food industry.
Due to the fact that whey proteins have much higher digestibility than casein, they are often used in production of infant formulas and to improve the nutritional value of dairy products but also of many other food products. Also, it is important to mention immunoglobulins and other glycoproteins (lactoferrins, transferrins) and enzymes (lysozyme, lactoperoxidase) which are very notable factors of immunoactive system of whey. They have antimicrobial properties and have the ability to reduce or even inhibit allergic reactions (Tratnik, 2003).

However, lactose is the main constituent (about 70%) of the whey dry matter and is a very important source of energy, but it has several roles. Some of beneficial effects of lactose are stimulation of peristaltic activities in the digestive tract, alleviation of calcium and phosphorous absorption, establishment of mild acidic reaction in the gut, which inhibits the growth and proliferation of pathogens. Furthermore, lactose also assures the optimal amount of magnesium and improves digestion of milk fat and other nutrients in human body and it does not participate in plaque formation. Heat treatments of whey cause transformation of certain
amount of lactose into lactulose, which promotes the growth of bifidobacteria (Tratnik, 2003).

Water soluble vitamins present in milk also pass into the whey, but their amounts vary and highly dependent on the storage conditions of whey. The whey is a good source of riboflavin, folic acid and cobalamin. The latter ones are bounded to whey proteins and mostly remain in whey after cheese manufacture. It is interesting that whey can contain higher amounts of riboflavin than milk, due to the activity of some lactic acid bacteria used in cheese manufacture. Due to relatively high content of riboflavin, whey has a characteristic yellow-green colour (Popović-Vranješ and Vujičić, 1997; Tratnik, 1998).

Mineral composition in whey varies to a great extent (7-12% dry matter) and depends on the technological process of cheese production (Popović-Vranješ and Vujičić, 1997). Whey contains almost all soluble salts and microelements present in the milk and also the salts added in the cheese manufacture process. Thereby calcium and phosphates are partially remaining bounded in the casein contained in cheese, and their
contents are much higher in acid whey because of much higher solubility in acid medium (Tratnik, 1998).

**2.4.2. Various types of beverage from whey:**

Researchers have used various types of whey for beverage preparation. For instance an investigation was conducted on utilization of pomegranate juice for the preparation of Chakka whey beverage (CWB), with a view to assess the possibility of Chakka whey beverage using pomegranate juice in the preparation of beverage. Pomegranate juice @ 0 (T₁), 10 (T₂), 15 (T₃) and 20 (T₄) per cent (v/v) with 10 per cent sugar was mixed in chakka whey for manufacture of beverage. Different levels of pomegranate juice had a definite effect on improving the sensory quality of the beverage. The beverage prepared by utilizing chakka whey with 15 per cent pomegranate juice (T₃), had secured the highest sensory score (8.81) and ranked as most acceptable product followed by T₂ with 8.65 points sensory score. The percentage of protein, total sugar, ash and acidity (%LA) of the product increased with increase in the levels of pomegranate juice (Babar *et al.*, 1971).
Cheddar cheese whey was used by Laonipon and Cardwell (1971), Kriel and Tonder (1979), Vieira et al., 1986; Jayaprakasha et al., 1986; Reddy et al., 1987; Singh et al., 1994; Khamrui and Rajorhia (1998b); Suresha and Jayaprakasha (2004).

There is a growing interest in utilization of whey byproducts. The study was carried out to develop a cultured milk beverage using Cheddar cheese whey. The ingredients used were cheddar cheese whey, mango pulp, sugar, full cream milk powder and yoghurt culture (DVS) and the role of each ingredient in the processing of the product was examined. Shelf life of most sensory scored sample was determined by evaluating chemical and microbial properties. According to the two factor factorial model 9 samples were obtained by mixing different levels of ingredients. A sensory evaluation was carried out to detect the appropriate levels of ingredients in the final product. Data were collected from 9 samples and were analyzed by using the Friedman test of the Minitab 15 software. Treatment with 1L of whey, 100ml of Mango pulp, 180g of sugar and 40g of milk powder exhibited the highest overall
acceptability with 11 days of shelf life. There was no treatment effect on viscosity. (Senarathna, et al., 2009).

Cottage cheese whey (Crippen and Jeon 1984; Chen et al., 1979; Bernal and Jelen, 1989) was used for beverage preparation. Chhana whey (Gagrani et al., 1987; Krishnaiah et al., 1991; Mann, 1996; Mandal et al., 1997; Kar et al., 1998; Prasad and Sharma, 2002; Saravana and Manimegalai, 2003, Maity et al., 2008) and Paneer whey (Singh et al., 1994; and Singh et al., 1999) was effectively utilized.

Attempts were made by Divya and Archana Kumari (2009) to develop a soft beverage from Paneer whey and guava pulp which pasteurized at different temperatures and timings for estimating its shelf-life. In the preparation of beverages the volume of guava pulp (25%), sugar (10%) and Paneer whey (65%) were kept constant while the pasteurization temperatures and timings were varied from 60°C-70°C for 15-35 minutes. The prepared beverages were evaluated for their physico-chemical properties and organoleptic qualities every 15 days till 45 days. Effect of different temperatures, timings and storage periods on the mean
sensory score of whey-guava beverage was significant and significant changes were observed in total sugars, reducing sugars, non reducing sugars and vitamin C during the storage period. However, whey-guava beverages pasteurized at 70°C for 35 minutes was found to be best in terms of sensory quality after 45 days and pH, acidity, protein, total sugars and reducing sugars found to be high than that of the other samples.

According to Yang and Silva (1995), fermentation is a means of modifying the functional properties of whey to add value. During fermentation, complex whey nutrients are converted into simpler forms, which can easily be assimilated in the intestine. Fermentation by probiotic bacteria increases the amount of easily digested amino acids, making fermented dairy products a good source of nutrients during diarrhoeal episodes (Hitchins and McDonough, 1989). Dietary calcium, in the form of calcium phosphate selectively favours the growth of intestinal lactobacilli and decreases the severity of *Salmonella* infections in rats (Bovee-Oudenhoven *et al.*, 1997; Bovee-Oudenhoven *et al.*, 1999).
The metabolic activity of the participating microorganisms results in production of various compounds. Due to the formation of certain acids and flavouring compounds, fermented whey products are more palatable as compared to non-fermented ones. Organic acids present in fermented dairy products are considered to have an important role in the health benefits. Fermentation of carbohydrate and protein components give rise to short-chain fatty acids (SCFA).

The SCFA such as acetic acid, citric acid and lactic acid have been reported to have antimicrobial activity against *E. coli* in the intestine (Sinha, 1986). The low pH of these products helps in the secretion of bile juices, absorption of nutrients and may reduce the concentration of pathogenic micro flora in gut. The general hypothesis is that the SCFA may be used in the colon to promote water and electrolyte absorption, just as glucose does in the small intestine (Desjeux, 2000), a situation required for diarrhoea treatment.

Fermentation results in many biologically active peptides that enhance the functionality of fermented foods/drinks. Finally, pleasant flavour formed during
fermentation, masks the original cheesy whey flavour (Jajoria, 2004). Shilovskaya (1981) found that whey fermented with *L. acidophilus* and enriched with amino acids, lactic acid salts, and antibiotic substances was effective as a prophylactic and therapeutic agent. When pigs were reared on milk whey inoculated with a probiotic culture of *L. acidophilus* and *Kluvyeromyces fragilis* an improved health status along with reduction in GI disorders was observed (Saara *et al.*, 1986). Gritsenko *et al.* (1989) used *L. acidophilus* strain 43S for fermenting salty whey and found that the fermented salty whey had an antagonistic action against *E. coli*.

*L. acidophilus (Johnsonii)* La1 has been shown to effectively suppress the growth of *Helicobacter pylori* in *vitro*. Using a drinkable, whey-based *L. acidophilus (Johnsonii)* culture, volunteers were found to exhibit a marked decrease in hydrogen breath test values of 9.4 vs. 20.4 in controls (*p*<0.03) (Michetii, 1999).

**2.5. Treatment of whey to be utilized for preparation of beverages:**

As whey has a wide range of essential compounds for human nutrition, high nutritional value, favorable nutrient density and good stability, it offers beneficial
applications in food and dairy industry. However, the limiting aspects of whey are: a bad image in relation to human food, unpalatable, unarguable consistency and high mineral contents.

In general whey from any source may be used i.e. sweet whey, sour whey or powdered whey, but use of powdered whey makes the final product unnecessarily expensive (Prendergast, 1985). Whey or whey derivatives derived from cheese, casein, Paneer etc. can be used for beverage preparation.

Several group of workers namely Brunner et al., (1969); Nelson and Brown (1969); Nelson et al., (1972); Kriel and Tonder, (1979); Vasileva and Zyuzkova (1982); D'yachenko and Suarez (1984); Kravchenko (1988); Vieire et al., (1986); Mann (1987); Mathur et al., (1988); Singh et al., (1994); Singh et al., (1999); Khamrui and Rajorhia (1998 b); Saravana and Manimegalai (2003) have utilized whey as such (without deproteinization) for beverage preparation.

For increasing the utility of whey, various technological modifications were carried out such as deproteinization, lactose hydrolysis etc. Deproteinization is carried out for imparting an attractive appearance to beverage as whey proteins display minimum solubility in acidic pH range and result in cloudy or sediment formation. Deproteinization of whey can be carried out by various methods such as heat, acid precipitation, ultrafiltration, cold precipitation etc. Among these, now a day in western countries ultrafiltration (UF) is widely used for manufacture of whey protein concentrates (WPC).

Many other research workers carried out the deproteinization by employing heat treatment to whey [Holsinger et al., (1974); Francis and Ann, (1979); Crippen and Jeon (1984); Jayaprakasha et al., (1986); Gagrani et al., (1987); Reddy et al., (1987); Krishnaiah et al., (1989); Krishnaiah et al., (1991); Mann, (1996); Mandal et al., (1997); Kumar et al., (2001); Prasad and Sharma (2002)].

Developments in membrane technology enable to separate the whey proteins and uses of ultrafiltred whey permeate for beverage preparation. Modification in
beverage preparation by using UF-whey permeate have been carried out by many workers [Francis and Ann (1979); Anon (1983); Kravchenko (1988); Vieira et al., (1986); Mann (1987); Vojnovic et al., (1993); Mann (1997); Suresha and Jayaprakasha (2003, 2004); Sheetal Deosarkar (2004)].

Use of lactose hydrolyzed whey is one of the modifications in beverage preparation. An attractive way of improving the attributes of whey, as a beverage base is to hydrolyze its lactose to sweeter syrup consisting of glucose and galactose, as both of them are sweeter than lactose. Many research workers tried the lactose hydrolyzed whey for beverage preparation (Francis and Ann 1979; Crippen and Jeon, 1984; Kravchenko, 1988; Bernal and Jelen, 1989; Paul, 1990; Driessen and Berg Vanden, 1990; Mann, 1996; Kar et al., 1998; and Suresha and Jayaprakasha, 2004; Sheetal Deosarkar, 2004).

2.5.1. Pretreatments of whey:

Following are some of the pretreatments recommended for whey to be utilized for beverage preparation.
2.5.1.1. Clarification/Separation:

For making sparkling beverage, the whey should be clear and this can be achieved by removing suspended large casein particles by filtration, using double layered muslin cloth and fat by passing it through cream separator at 45°C (Singh et al., 1994).

2.5.1.2. Pasteurization/Heat treatment of whey:

The heat treatment is given to whey for its pasteurization. Several workers reported their work on pasteurization of whey, for instance pasteurization was carried out at 70°C/5 min (Kriel and Tonder, 1979), 85°C (Prendergast, 1985), 74°C/16 sec (Mann, 1987), 72°C/15 sec (Khamrui and Rajorhia, 1998b) and 70±1°C/30 min (Singh et al., 1994).

2.5.1.3. Deproteinization of whey:

Whey proteins displays minimum solubility in acidic pH range employed for beverage preparation and results in cloudy or sediment formation. For imparting an attractive appearance to beverage, effective clarification is essential. Crippen and Jeon (1984) deproteinized whey by heating to 90°C/10 min, filtering or centrifuging to remove coagulated protein, pH
increased to 5.6 using calcium hydroxide and further increased to 6.5 by adding potassium hydroxide. It was again filtered and centrifuged.

Heating of clarified whey to 98°C for 10 to 30 min and addition of CaCl2 (0.2-0.5%) provide maximum clarification. Mathur et al., (1986) indicated that addition of CaCl2 could be useful for heat clarification of whey at pH 5.5. Thermo-chemical precipitation of whey protein, cooling and separation by centrifugation and filtration is generally employed.

The whey was deproteinized by heat treatment at temperature 90°C for one hour and kept undisturbed overnight at refrigeration temperature to allow the precipitated protein to settle down. The whey was filtered using sterilized cotton padded muslin cloth to obtain deproteinized whey (Reddy et al., 1987). Gagrani et al., (1987); Krishnaiah et al., (1989, 1991); Mandal et al., (1997) and Prasad and Sharma (2002), separated two third of fat in whey by using cream separator and the fat free whey obtained was boiled for 30 min. The boiled whey was kept undisturbed overnight at room temperature to allow the precipitated protein to settle down. Next day morning the whey was filtered through
muslin cloth to obtain deproteinized whey. Cold precipitation by employing sodium hexametaphosphate (SHMP) @ 0.2-0.5% was observed to provide excellent clarity at pH 2.5 (Mathur et al., 1986). Furthermore, SHMP could be employed more effectively for clarification of whey without application of heat and thus preserve some of heat liable nutrients of whey. Separation of proteins by employing ultrafiltration and use of permeate as base for beverage manufacture were also tried (Vieire et al., 1986; Mann, 1987; Kravchenko, 1988; Paul, 1990 and Vojnovic et al., 1993). Ultrafiltration of whey, after pasteurization and cooling to 50°C was carried out at 100 psi pressure to get deproteinized whey i.e., whey permeate (Suresha and Jayaprakasha, 2003, 2004).

2.5.1.4. Lactose hydrolysis:

Lactose, the major component of whey is a disaccharide with component monoglyceride, viz., glucose and galactose joined together in β-1-4 linkage. An attractive way of improving the attributes of whey as a beverage is to hydrolyze its lactose to sweeter syrup consisting of glucose and galactose, as both the components are sweeter than lactose. Acid hydrolysis
and enzymatic hydrolysis are two methods of lactose hydrolysis. The first method is characterized by very severe pH and temperature conditions (pH=1-2, T=100-150°C), thus rendering the end product unsuitable for use as a food ingredient and it is suitable only for hydrolysis of lactose in absence of protein whereas enzymatic may be suitable for whole whey as well as deproteinated whey. Enzymatic hydrolysis of lactose using β-D galactosidase seems to be an attractive method. Simplest method of achieving lactose hydrolysis by enzyme is by direct addition of lactase but disadvantage is that large amount of expensive enzyme is required and it could affect the properties of product as enzyme is lost in to the product. Other method of delivering enzyme economics involves retaining enzyme within an UF reactor, immobilizing it on solid support or entrapping enzyme within cellulose triacetate fibers (Delaney, 1981; Ryder, 1986).

A whey-based drink, Lactofruit was prepared by hydrolyzing the deprotenized whey to 50% with lactase enzyme (Francis and Ann, 1979). Athletic type of drink was prepared from hydrolyzed cottage cheese whey. The processing steps include deproteinization (heating to
90°C, followed by addition of calcium hydroxide at 0.10 to 0.15 g/100 ml of filtered whey and stirring for 15 min, which resulted in pH of 5.6 to 5.7. The pH was then raised to 6.5 with saturated potassium hydroxide and again it was filtered to remove the cloudiness caused by calcium hydroxide) and hydrolyzed using Maxilact L 2000 (β-D-galactosidase, GB Fermentation Industries, Inc., Charlotte, NC) at a level of 0.085 % (v/v) and at 5°C for 18 h (Crippen and Jeon, 1984).

Soft drink 'Natures Wonder' was developed by Swedish Dairy Cooperative Arla Group, in which four part of the hydrolyzed whey permeate were blended with six parts of juice consisting of orange and pineapple juices (Kravchenko, 1988). Bernal and Jelen (1989) used the hydrolyzed cottage cheese whey for development of whey drink. They used enzyme derived from Kluyveromyces marxianus var. lactis in one batch and Aspergillus oryzae in other. The condition for first was 38°C and pH 6.8 whereas for second it was 55°C and pH 4.65. The pH adjustment was made with 5.0 M hydrochloric acid or potassium hydroxide. Kar et al., (1998) hydrolyzed the whey using Maxilact from Saccharomyces lactis, 0.3 g/lit, resulting in 80%
hydrolysis in 30 min for manufacture of fermented whey drink.

Zadow (1986) reported an optimum temperature of 35°C for lactase enzyme derived from *Kluyveromyces lactis*. A fermented drink was made by hydrolyzing the lactose using 0.25-1.0 g/lit of lactase enzyme (Paul, 1990). Jelen (1992) reported that neutral lactases have optimum activity at 36-38°C. The hydrolysis of 80% was obtained at 37°C after 1 h incubation when Lactozyme added at the rate of 1.0 ml/lit (Suresha and Jayaprakasha, 2004).

2.6. Improving the quality of whey-based beverages by various adjuncts:

2.6.1. Sugar:

The basic composition of fruit based drinks is determined by sweetness-to-acid ratio, which is dependent upon the type of flavour and product in question (Gatenby, 1993).

Research workers tried different levels of sugar for instance Nelson et al. (1972) and Gagrani et al., (1987) found that sugar level of 18-20°Brix provides the acceptable quality beverage. Pasteurized sugar syrup
(15%) was used by Holsinger et al., (1974). Whey beverages with highest sensory score were prepared using sugar level from 6-10% (Mathur et al., 1988; Paul, 1990 and Singh et al., 1999). An alcoholic drink 'Wheyvit' was prepared using 22-23% of 50% sugar solution (Bambha et al., 1975). The fermented beverage prepared by Gandhi (1989) had 8-10% sugar level. Orange flavoured whey beverage was prepared using sugar level of 5% (Francis and Ann, 1979), 9% (Mann, 1987) and 7-10% (Vojnovic et al., 1993).

The whey beverages with lemon flavour were prepared using sugar in the range of 5 to 14% (Reddy et al., 1987; Singh et al., 1994; Mandal et al., 1997 and Prasad and Sharma, 2002). Krishnaiah et al., (1991); Singh et al., (1994) and Saravana and Manimegalai (2003) used sugar @ 7 to 10% in pineapple and banana whey beverages. The mango based whey beverage with acceptable quality was prepared using 7% (Singh et al., 1994) and 4% (Mann, 1996) sugar addition.

The chocolate whey drink prepared by Kriel and Tonder (1979) and Vieire et al., (1986) contained 5% and
6% sugar, respectively. Kinnow based ready-to-serve (RTS) beverage preparation contained 7% sugar (Khamrui and Rajorhia, 1998b).

The lactose hydrolyzed (80% hydrolysis) whey beverage were prepared with 4% sugar level compared to 7% sugar in unhydrolyzed samples (Bernal and Jelen, 1989). Suresha and Jayaprakasha (2003 and 2004) prepared a beverage from ultrafilterated whey permeate using 10% sugar in case of unhydrolyzed permeate whereas 8% sugar in case of hydrolyzed permeate beverage. Addition of honey to such beverage instead of sugar or other sweeteners results in fortifying it with numerous other nutrients like vitamins, minerals (Hammond, 1992) and phytochemicals which are not naturally present in whey (Jelićić et al., 2008)

2.6.2. Addition of flavourings/fruit pulp:

The demand for any fruit based product is largely based on their flavour, colour and appearance and nutritive value. These quality factors are directly dependent on structure and chemical composition of fresh fruit (Nelson et al., 1972). There are vast range and variable amount of fruit juices or pulps were utilized for development of acceptable whey based
beverage (Srivastava and Kumar, 1994). Many fruit juices are either too acidic or too strongly flavoured to make pleasant beverages (Khamrui and Rajorhia, 1998a).

Fruit juices contain highly odorous and volatile flavours, which get lost during, heat processing and so retention and stabilization in final product is done by adding flavour. The natural colour tends to fade during heat processing and storage therefore food grade synthetic colour was added by these workers.

The main purpose of adding flavour or fruit pulp is to mask the unpleasant characteristics of whey taste and flavour and increase its palatability and nutritive value with quality improvements. Juice mixes may be fruit juice and/or fruit pulps, which are generally sweetened, flavoured, acidified, coloured and sometimes chemically preserved. They are usually formulated from fruit juices, milk solids, sugar, acidulant, buffering agents, stabilizers, emulsifiers, flavouring and colouring ingredients, preservatives and nutrients (Lang, 1969; Mann, 1994; Ibrahim et al., 1993a,b).
Different types of fruit juices/pulps at various levels were tried for improving the quality of whey beverages. Mann (1987) and Vojnovic et al., (1993) used 9-40% orange juice in whey beverage. Orange juice concentrate could also be used (Brunner et al., 1969; Laonipon and Cardwell, 1971; Francis and Ann, 1979 and Marhounova, 1980). Crippen and Jeon (1984) used 0.2 % (v/v) orange flavour. Reddy et al., (1987), Prasad and Sharma (2002) and Mandal et al., (1997) added lemon juice at various levels. Nelson et al., (1972) developed flavoured drinks containing peach puree (20%), strawberry (10%) and raspberry (10%) and orange flavouring and observed that orange flavoured drinks were highly acceptable.

Chocolate whey beverage was prepared with cocoa and 0.1% vanilla chocolate (Kriel and Tonder, 1979 and Vieira et al., 1986). Vasileva and Zyuzokova (1982) prepared the delicious beverage containing blackthorn juice. Gagrani et al., (1987) used various flavours such as orange (10%), pineapple (15%), guava (25%) and mango (15%) for providing acceptable beverage. Pineapple, mango and orange essence at various concentrations (0.25, 0.30, 0.35, 0.40 and 0.45 ml/lit)
were tried by Suresha and Jayaprakasha (2003) and observed that pineapple (0.40 ml/lit), mango (0.35 ml/lit) and orange (0.35 ml/lit) were most preferred in this order.

Different essences namely orange, pineapple and banana (Krishnaiah et al., 1989 and 1991) as well as mango pulp (15%) and pineapple juice (20%) were tried (Singh et al., 1994). These workers found that mango flavour was preferred by most of the consumers. Kinnow based RTS beverage was manufactured by Khamrui and Rajorhia (1998b) having 40% of Kinnow juice. Shukla et al., (2000) developed RTS beverage from whey by addition of 10-30% litchi juice.

A grape fruit juice was also used in whey beverage (Nelson and Brown, 1969; Bernal and Jelen, 1989 and Mann, 1996). Singh et al., (1999) tried various levels of guava extract : whey (1:5, 1:4 and 1:3) and it was observed that 1:3 levels were most suitable. An acceptable fermented beverage can be prepared by using banana and whey in proportion of 75:25 (Kumar et al., 2001).
Besides fruits, some scientists have studied the suitability of addition of other flavouring agents like chocolate, coca, vanilla, cereals (mostly rice, oat and barley), honey, etc. Addition of cereals, especially bran, seems to be very interesting and it resulted in production of a very good beverage fortified with dietary fibers, essential fatty acids (with addition of oat) and hypoallergenic proteins which makes these beverages suitable for consumption by allergic population and children. In order to prepare a hypoallergenic beverage, the addition of other vegetable sources of proteins like isolates of potato or soy proteins may be used. Fortification of such juices with oatmeal is preferred because it not only enhances the low allergenic protein content, but also adds to the taste of the resulting product (Girsh, 2001).

Addition of many other fruits like concentrates of apple, pear, peach, apricot and cherry has also been applied. The addition of berries which are known as a good source of iron and antioxidants have proved to be very useful. That is especially important in production of whey beverages with improved nutritional value. Best
example for supporting these hypotheses is Brazilian group of scientists who have developed a whey drink flavoured by addition of strawberry concentrate and fortified with ferrous biglycinate. They have proved that long-term consumption of this drink had an impact on reduction in the prevalence of anemia in children and adolescents (Miglioranza et al., 2003).

In last two decades numerous patents containing recipes for production of whey beverages with addition of fruit concentrates with variable fruit dry matter amounts (5-20%) have been registered. Thereby citrus-flavoured drinks and drinks with addition of other tropical fruit aromas like mango, banana or papaya have been most frequently suggested, since they have proved to be very efficient in covering up the undesirable odour of cooked milk and salty-sour flavour of fresh whey (Đurić et al., 2004). However, the main problem occurring in all these recipes (especially when adding fruits like apples, pears and bananas) is the formation of sediment due to the high amount of fruit dry matter and interactions of proteins with components in fruit dry matter (Jeličić et al., 2008).
2.6.3. Colorants:

Among the food grade colours rose, orange and lemon were tried by Singh et al. (1999) and it was observed that lemon colour was most preferred with guava extract. Krishnaiah et al., (1989,1991) used yellow, lemon and orange colour with pineapple, banana and orange essence, respectively.

Suresha and Jayaprakasha (2003 and 2004) used various colours namely lemon, yellow, saffron and orange red at various concentration (0.25, 0.30, 0.35 and 0.40 ml/lit) and observed highest score at the concentration of 0.30 ml/lit of beverage.

2.6.4. Acidulants:

Acidification of beverage is important to enhance flavour and to protect the product from microbial deterioration by preventing microbial growth (Khamrui and Rajorhia, 1998a). Citric, tartaric and malic are important natural acids of fruits and citric being the most widely employed in the formulation. Drinks having pH 3.8-4.2 are reported to be lighter and less astringent (Prendergast, 1985). A pH less than 4.0 was necessary to prevent protein coagulation during pasteurization.
Beverages were adjusted to different pH that is 3.6 (Nelson et al., 1972), 3.7 (Crippen and Jeon, 1984), 3.8 (Laonipan and Cardwell, 1971), 4.25 (Khamrui and Rajorhia, 1998b) and 5.7, 5.8 (Marhounova, 1980). A pH of 4.5 was adjusted in case of mango and pineapple and 4.0 in lemon whereas 5.0 in case of banana (Singh et al., 1994).

Citric acid at various levels ranging from 0.1 to 1.0% of finished product was tried by many research workers (Mandal et al., 1997; Prasad and Sharma, 2002; Suresha and Jayaprakasha, 2003, 2004) and obtained at 0.10% best results with desirable tartness.

2.6.5. Preservatives:

Microbial activity in juices and beverages is usually controlled by adjustment in pH value to lower than 4.0 which is almost 100% effective against pathogens (Batchelor, 1993). Sugar content in excess of 65% will provide protection against yeast by lowering the water activity (Khamrui and Rajorhia, 1998a). According to Fruit Products Order (FPO, 1955), chemical preservatives permitted to be used in fruit products are: sulphur dioxide (including potassium metabisulphite)
and benzoic acid (including benzoates). Potassium metabisulphite has good preservative action against moulds and pathogens and it inhibits enzymes also, whereas, benzoic acid has a low taste threshold, low volatility and wide anti-microbial spectrum (Khamrui and Rajorhia, 1998a). These two chemical preservatives are widely used throughout the world in the preservation of juice, pulp, nectar, squash, crush, cordial, and other fruit products (Jay, 1996).

The FPO (1955) allows the addition of sulphur dioxide up to a maximum level of 700 ppm in fruit juice, 350 ppm in squash, and 100 ppm in RTS beverages. The permitted level of benzoic acid is 100 ppm and 600 ppm in RTS beverages and squash, respectively.

Amongst the addition of sodium propionate (0.05 %) along with potassium metabisulphite (0.01 %) and sorbic acid (0.06 %) in beverage, sorbic acid was found more effective and product had storage life of 35 days at room temperature (Mandal et al., 1997). Sodium benzoate @ 300 ppm was used as preservative by Singh et al., (1999).
The lactose hydrolyzed whey beverage subjected to storage studies with the addition of two different preservatives, namely, sorbic acid (SA) (@ 0.06 %) and sodium benzoate (SB) (@ 0.03 %) under refrigeration conditions (7±10°C) along with control (without preservative). The SPC and yeast and mould count (Log cfu/ml) was studied during the storage and it was observed that SPC increased as storage advanced. The control had significantly higher SPC compared to beverage containing either SB or SA on 14th day of storage. During 35 days of storage, the increase in count was faster in beverage containing SA than SB as preservatives. The yeast and mould count also showed similar trend, with high counts were found in control up to 14 days of storage. During further study up to 35 days, the count was increased significantly in beverage containing SA than SB. Hence, SB was found more effective as preservative compared to SA (Shital Deosarkar, 2004)

2.6.6. Stabilizers:

Different types of stabilizers namely pectin, sodium alginate, carrageenan, carboxy methyl cellulose (CMC), guar gum, etc., are used to control
sedimentation (Jelen, 1992; Mann, 1994). They are generally added in the range of 0.05 to 0.1 % (Paul, 1990; Khamrui and Rajorhia, 1998a and Puranik, 1999). Medium esterified pectin and cereal extract (Francis and Ann, 1979), guar gum (Kriel and Tonder, 1979; Vieira et al., 1986) and Frimubion MD (Marhounova, 1980) were used in whey drinks. Khamrui and Rajorhia (1998a) added 0.15% CMC and 0.08% pectin. Chocolate whey drink was prepared with carrageenan and carboxy methyl cellulose (1:3) (Driessen and Berg Vanden, 1990).

2.7. Post-processing treatments of whey beverages:

The quality of the final product depends upon the treatments given during its manufacturing. During whey beverage preparation, heat treatments involved are 75°C/30 min (Bambha et al., 1975), 90°C (Marhounova, 1980), 90°C for 10 sec (Khamrui and Rajorhia, 1998a), 80°C/20 min (Kriel and Tonder, 1979) and 72°C/16 min (Vieira et al., 1986 and Mann, 1987). They have also carried out homogenization of beverage at the pressure 20 Mpa to 21,000 Kpa followed by hot filling. Krishnaiah et al., (1991), Suresha and Jayaprakasha (2003) and Saravana and Manimegalai, (2003) followed in-bottle
heating/sterilization at 110°C/15 min, 80°C, respectively and subsequently cooled to room temperature and stored at 4°C. Carbonation was performed by former group of scientists. The filled product was stored at various low temperatures ranging from 4 to 8°C.

Lactose hydrolyzed whey beverage prepared by Crippen and Jeon (1989) was heated at 88°C and bottled. The bottles were capped, inverted and held for 5 minutes and then following turning and cooled to room temperature. Reddy et al., (1987) heated the beverage at 80°C/15 min followed by filtration and bottling, and sterilization (120°C/10 min) and storage (4-6°C). Homogenized, UHT processed and aseptically packed whey beverage with longer shelf life can be prepared (Mathur et al., 1988). Lactose hydrolyzed whey permeate beverage were pasteurized at 80°C (Suresha and Jayaprakasha, 2004).

The beverages were filled in sterilized glass bottles (Bambha et al., 1975; Vieira et al., 1986; Mann, 1987; Crippen and Jeon, 1989; Reddy et al., 1987; Saravana and Manimegalai, 2003 and Suresha and Jayaprakasha,
2004), cartons (gable-top paper board) with foil lining (Kravchenko, 1988) and pouches (Paul, 1990).

2.8. Shelf life of the whey-based beverages:

Shelf life of any food product is most important from manufacturer as well as consumers' point of view. The shelf life of food products is governed by ingredients used, processing conditions, preservatives used, packaging, storage conditions, etc. The purpose of heating/sterilization is to make beverage pathogen free for consumers' health point of view and increase its shelf life. Whey beverage with shelf life of 6 month without refrigeration was obtained by heating at 90°C and packing aseptically into Tetra pack (Holsinger et al., 1974).

In general shelf life of different whey beverages reported were 21 days at 8°C (Marhounova, 1980); 8 weeks in aseptically packed beverage (Kriel and Tonder, 1979); 30 days to 6 months at 4-6°C (Vieira et al., 1986; and Mann, 1987); 6-8 weeks (Prendergast, 1985); 4 months at 8±1°C and 1 month at 37°C (Mathur et al., 1988); 2 to 3 weeks at 5±1°C (Paul, 1990); 45 days at 4°C (Saravana and Manimegalai, 2003); and 15 (non-
carbonated) and 30 days (carbonated) at 4°C (Suresha and Jayaprakasha, 2003).

Mandal et al. (1997) prepared three types of beverages, which were stored at room temperature with or without preservatives (Sodium propionate, 0.05% and Sodium metabisulphite, 0.01%) and at refrigeration temperature for a period of 45 days. They observed all beverages were acceptable with identical organoleptic quality for a minimum period of 35 days under all storage methods. These workers also tried combination of sodium propionate (0.05%) and potassium metabisulphite (0.01%) in first lot and sorbic acid (0.06%) in second lot. It was then sterilized in an autoclave at 110°C/15 min and crown corking under aseptic condition utilizing laminar flow workstation. Sorbic acid was found more effective and yielded the beverage with the storage life of 35 days at room temperature. Divya and Archana Kumari (2009) found that 70°C for 35 min was best in terms of sensory quality after 45 days and pH, acidity, protein, total sugars and reducing sugar was higher than other samples.
2.9. Probiotics as functional ingredients:

One widely used definition, developed by the World Health Organization and the Food and Agriculture Organization of the United Nations, is that probiotics are "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host."

Probiotics are commercially available in foods and dietary supplements (for example, capsules, tablets and powders) and in some other forms as well. Examples of foods containing probiotics are yogurt, fermented and non fermented milks, miso, tempeh and some juices and soy beverages. In probiotic foods and supplements the bacteria may have been present originally or added during preparation. Most probiotics are bacteria similar to those naturally found in human guts, especially in those of breastfed infants (who have natural protection against many diseases). Most often, the bacteria come from two groups, Lactobacillus or Bifidobacterium. Within each group, there are different species (L. acidophilus and Bif. bifidum) and within each species, different strains or varieties. A few common probiotics,
such as *Saccharomyces boulardii* are yeasts, which are different from bacteria. Another development, which although not strictly covered by the definition of probiotics, but is conceptually related to selectively stimulate the growth of a target population of desirable bacteria, already present in the gut, particularly, the colon. The approach has been used for many years in the form of lactulose supplementation, but recently the focus has shifted to oligosaccharides based on various sugars. The substances (eg. fructo-oligosaccharides, xylo-oligosaccharides, lactulose, etc.), which on consumption increase the incidence and numbers of bifidobacteria in faeces have been named "bifidogenic factors" (Modler, 1994).

2.10. Effect of intrinsic and extrinsic parameters on probiotic microorganisms:

The growth patterns of probiotic organisms are not uniform. Several intrinsic and extrinsic parameters affect the growth of probiotics. Following is the summary of work conducted by various workers on this important aspect.
2.10.1. Effect of pH:

Laroia and Martin (1990) reported that bifidobacteria die quickly at low pH values (<pH 5.5) since they cannot tolerate prolonged exposure to acidic conditions. Growth of most strains is retarded below pH 5.0 (Shah, 1997). Good survival of bifidobacteria during refrigerated storage (5-7°C) in fermented milks is an exceptional property that varies from strain to strain (Martin and Chou, 1992; Dave and Shah, 1997). The pH was also found to affect the viability of *L. acidophilus* (Dave and Shah 1997). Hence, tolerance to pH and acidity must be considered while selecting probiotic strains for incorporation into fermented milk products (Klaver *et al.*, 1993). The increased buffering capacity of highly concentrated (5:1) ultra filtered milk allows higher developed acidity, for a given pH (Ventling and Mistry, 1993).

Modler and Villa-Garcia (1993) are of the view that frozen yoghurt with a titratable acidity of 0.25 to 0.32% lactic acid is a better suited carrier food for the probiotics than freshly cultured yoghurt, which has an acidity of 1.02-1.4 % lactic acid. The technique of encapsulation with milk fat used in their study was
ineffective in preventing acid injury to the bifidobacteria.

Samona and Robinson (1994) found that a pH above 4 units had no marked effect on the viability of three strains of bifidobacteria tested. Indeed, all samples of yoghurt that contained insufficient numbers of probiotic bacteria had a pH of less than 4 (Rybka and Fleet, 1997).

2.10.2. Effect of low temperature treatment:

Hughes and Hoover (1995) reported good survival and enzyme stability in four strains of bifidobacteria and one strain of *L. acidophilus* during refrigerated storage in fermented milk. Bifidobacteria were significantly less tolerant to low temperature than *L. acidophilus*. Storage temperature of 10°C affected the viability of bifidobacterium species in probiotic yoghurt and the counts of *L. acidophilus* did not show significant variation (Dave and Shah, 1997).

Rybka and Kailasapathy (1995) studied the survival of these bacteria in freeze-dried yoghurt powder and in the reconstituted product. The number of viable bacteria per unit of reconstituted lyophilized yoghurt powder was
lower by up to three log cycles than the fresh liquid product due to metabolic injury during freeze drying (96 h at -50°C) and storage at room temperature under anaerobic conditions for two and a half months.

2.10.3. Effect of oxygen content:

Oxygen content and redox potential affect the viability of bifidobacteria during the manufacture and storage of dairy products. Exclusion of oxygen requires special equipment, which may be very costly (Ishibashi and Shimamura, 1993; Klaver et al., 1993). Oxygen permeation through packages is also recognized as an important factor. Dissolved oxygen content and the redox potential increased gradually during storage of probiotic yoghurt in plastic cups (Dave and Shah, 1997). Bifidobacterium species and _L. acidophilus_ showed improved survival when the product was prepared and stored in glass bottles. Viability of both _L. acidophilus_ and bifidobacterium species improved when the dissolved oxygen content in the product was low. Packages laminated with aluminium and complexes of polystyrene and polyethylene may act as good oxygen barriers and have been suggested for this purpose (Odet, 1995).
2.10.4. Effect of level of inoculums:

Reducing the level of inoculum of commercial probiotic yoghurt cultures resulted in slightly higher post acidification, which had adverse effects on the viability of probiotic organisms. Of the four organisms, *L. delbrueckii*, ssp. *bulgaricus* lost viability most rapidly with counts dropping below $10^5$ cfu/g after 20 days of storage at 4°C followed by *L. acidophilus* whose counts were satisfactory ($>10^6$ cfu/g) up to 25 days of storage after which a sharp decline was observed. The bifidobacterial counts were more satisfactory ($>10^8$ cfu/g) up to 35 days in the case of three yoghurt starter cultures than the lactobacilli. *Str. salivarius* ssp. *thermophilus* was most stable in yoghurt with counts exceeding $10^7$ cfu/g throughout the storage period for all levels of inoculum (Dave and Shah, 1997).

2.10.5. Effect of acidity:

Laroia and Martin (1990) reported that bifidobacteria die quickly at high acid values (<pH 5.5), since they cannot tolerate prolonged exposure to acidic conditions. Growth of most strains of bifidobacterium is regarded below pH 5.0 (Shah, 1997). Good survival of bifidobacteria during refrigerated storage (5 to 7°C) in
fermented milks is an exceptional property that varies from strain to strain (Martin and Chou, 1992; Dave and Shah, 1997). *Bif. adolescentis* is more frequently and more easily isolated from Australian yoghurts than other species of added bifidobacterium (Rybka and Fleet, 1997). The pH was also found to affect the viability of *L. acidophilus* (Dave and Shah 1997). Hence, tolerance to pH and acidity must be considered while selecting probiotic strains for incorporation into fermented milk products (Klaver *et al.*, 1993).

The increased buffering capacity of highly concentrated (5:1) ultra filtered milk allows higher developed acidity, for a given pH (Ventling and Mistry, 1993). Hence, it was felt that ultra filtered milks could be used for producing fermented milk products that contain bifidobacteria or for the production of an active bulk starter of bifidobacteria. The development of liquid or set yoghurt, using ultra filtered milk and a mixed culture of lactobacilli and bifidobacteria, was also suggested.

Modler and Villa-Garcia (1993) are of the view that frozen yoghurt, with a titratable acidity of 0.25 to 0.32 percent lactic acid, is a better suited carrier food for the
bifidobacteria than freshly cultured yoghurt, which has an acidity of 1.02 to 1.4 percent lactic acid. They observed a one log and three log decline in the viability of *Bif. longum* ATCC 15707 during 11 weeks of frozen storage of low acid (pH 5.85) and high acid (pH 4.47) yoghurts. Thawing and freezing of the mix resulted in an additional one log decline. The technique of encapsulation with milk fat used in their study was ineffective in preventing acid injury to the bifidobacteria.

However, Samona and Robinson (1994) found that a pH of above 4.0 units had no marked effect on the viability of three strains of probiotic bacteria tested. Indeed, all samples of yoghurt that contained insufficient numbers of probiotic bacteria had a pH of less than 4.0 units (Rybka and Fleet, 1997).

**2.11. Health benefits of probiotics:**

Probiotics are used in a wider range of health benefits ranging from the treatment of diarrhea (this is the strongest area of evidence, especially for diarrhea from rotavirus), to prevent and treat infections of the urinary tract or female genital tract, to treat irritable bowel syndrome (IDS), to reduce recurrence of bladder
cancer, to shorten how long an intestinal infection lasts that is caused by a bacterium called *Clostridium difficile*, to prevent and treat pouchitis (a condition that can follow surgery to remove the colon), to prevent and manage atopic dermatitis (eczema) in children, to treat various allergic syndromes and modulation of immune system.

The trend towards withdrawal of antibiotic use in animals has generated substantial interest in the application of probiotics. Ultimately, probiotics may also provide a better alternative to the use of oral antibiotics in the treatment of gastrointestinal disorders in human beings (Hull *et al.*, 1992). Future research on probiotic bacteria will, probably, center on the selection of new and more specific strains lactic acid bacteria. Different regions of the gastrointestinal tract may require different probiotic bacteria. Today, most probiotic strains are incorporated into dairy products. Functional foods of the future may include infant formulae, baby foods, fermented fruit juices, fermented soy products, cereal-based products and also disease-specific therapeutic foods. All these products will be required to
have good shelf-stability and a prolonged shelf-life (Lee and Salminen, 1995).

The health benefits of probiotics can be direct or indirect through modulation of the endogenous flora or of the immune system. Many health claims have been made concerning probiotics, especially concerning their potential to prevent or help cure gastrointestinal and related ailments. These include improved lactose digestion and other direct enzymatic effects, antibiotic-associated diarrhoea, prevention and curative treatment of gastroenteritis, constipation, intestinal infections and to get rid of pathogenic organisms colonized in gut, traveller's diarrhoea, irritable bowel syndrome and various conditions of diarrhoea, hypocholesterolaemea, urogenital diseases, atopic diseases, skin diseases, gastrointestinal well-being, inflammatory bowel disease and colon cancer.

These organisms possess various immunological functions Viz. mitogenic activity (Kado-oka et al., 1991), adjuvant activity (Kohwi et al., 1988), macrophage activation (Sekine et al., 1985), antibody production enhancement (Yasui et al., 1992), interferon production
(Kishi et al., 1996) and antitumor effect (Sekine et al., 1994). It was further indicated by a number of workers that both the cell wall and cytoplasm of the probiotic bacteria were found to have induced the mitogenic responses of spleen cells (Sekine et al., 1995; Hosono et al., 1997 and Vaughan et al., 1999). Health benefits of probiotic organisms related with gut microbiota are summarized in following paragraphs.

2.11.1. Probiotics in lactose intolerant individuals:

Many Asian people suffer from lactose intolerance due to a congenital deficiency of lactase resulting in the inability to digest lactose. It is known that these people can tolerate lactose in fermented milk products. Lactose is normally hydrolyzed into glucose and galactose in the small intestine. However, in case of people who are deficient in intestinal lactase, it passes into the large intestine, where it is fermented by putrefactive bacteria with the production of lot of gases, including hydrogen. Hydrogen enters the blood streams and is expelled through the respiratory system. Ingestion of milk and milk products by these individuals results in cramps, flatulence, diarrhoea and even damage of the intestinal
mucosa in children (Welch, 1987). Various workers reported a significant increase in lactose absorption in individuals who consumed milk containing $10^6$-$10^8$ cells of *L. acidophilus*/ml as compared to control groups consuming only milk (Kim and Gilliland, 1983 and Gilliland, 1985).

It was observed that the addition of a lyophilized preparation of *L. acidophilus* strain to milk, just before consumption was found to be associated with decreased symptoms in lactose maldigesting school children when compared with unsupplimented milk (Montes et al., 1995).

2.11.2. In constipation:

The problem of constipation in bedridden elderly people (average age 78 years) was found to be 10.8 times per 20 days, on an average. When given Bifidus yoghurt on a regular basis, the stool frequency increased to 13.1 times per 20 days and returned to the original level after the Bifidus yoghurt was discontinued (Tanaka and Shimosaks, 1982). Seki et al., (1978) demonstrated that cultured milk prepared using *Bif. longum* and *L. acidophilus* was more effective in improving stool regularity than milk cultured with *Str. thermophilus*.
alone. Intake of 130 g of bifidus yoghurt/day caused a 10-fold rise in the numbers of faecal bifidobacteria and an improvement of faecal character. Oral administration of *Bif. breve*-4006 (10⁹ to 10¹⁰ cfu/day) increased faecal counts of bifidobacteria and lowered faecal counts of bacteroides and enterobacteriaceae in rats and adult humans (Mada, 1981).

2.11.3. In stimulation of immune system:

Oral and intraperitoneal administration of cells of *L. acidophilus* cause macrophage activation and lymphocyte proliferation in mice. No difference was observed between the administration of viable and non-viable cells by means of the intra-peritoneal routes. However, in case of oral administration, the effect was more pronounced when viable cells were administered (Perdigon *et al.*, 1986, 1987, 1995). *L. acidophilus, L. casei, L. delbrueckii ssp. bulgaricus* and the yoghurt cultures were associated with a heightened systematic immune response in mice. Increase in the number of cells producing SIgA (Secretary Immunoglobulin A) was found to be dose dependent. The dose found to be 2.9X10⁹ cells per day for 2 days in the case of *L. casei* and *L. acidophilus* and for 7 days in the case of yoghurt.
culture. *L. casei* was found to be most effective. When cells of *E. coli* were administered either orally or intraperitoneally, it caused 64% mortality (Yamazaki *et al*., 1982).

### 2.11.4. Hypcholesterolaemic effect:

Hypercholesterolaemia is very widespread and is considered to be one of the leading causes of cardiovascular disease. The intestinal microflora may influence serum cholesterol levels (Gilliland, 1989, Khedkar *et al*., 1993). *L. acidophilus* apparently assimilated cholesterol from the growth medium while growing anaerobically in the presence of bile in vitro, and could be expected to do so in the intestine (Gilliland and Speck, 1977; Lichtenstein and Goldin, 1998).

Buck and Gilliland (1994) identified five isolates of *L. acidophilus* from the human intestines, that were better able to assimilate serum cholesterol and were more active at bile salt deconjugation than commercially used cultures of *L. acidophilus*. However, their ability to withstand production procedures needs to be ascertained, prior to use as dietary adjuncts.
Gopal et al. (1996) reported that cultures of *L. acidophilus* and *Bifidobacterium* spp. differ widely in their ability to deconjugate bile salts and lower cholesterol content in culture broth. They observed a weak positive correlation between the ability of Bifidobacteria to tolerate bile and lower cholesterol in the medium, but argued that the possibility of these organisms lowering serum cholesterol in vivo should not be discontinued without better studies, since transformation of cholesterol by certain microorganisms is well known.

2.11.5. Anti-carcinogenic properties:

The antitumor effects of administered Lactobacilli were reported by several investigators in mice (Goldin and Gorbach, 1976; Mada, 1981; Shahani et al., 1983 and Perdigon et al., 1995). Dietary supplementation with *L. acidophilus* delayed development of colon cancer in rats exposed to a chemical carcinogen (Goldin and Gorbach, 1980). Ingestion of Acidophilus milk in place of milk or unfermented acidophilus milk resulted in a reduced activity of faecal β-glucosidase and β-gluconidase enzymes that reportedly catalyze the
conversion of procarcinogens into carcinogens (Ayebo et al., 1980).

2.11.6. Antimicrobial activities of LAB:
Several workers through in vitro and in vivo investigations have reported antimicrobial effects of products containing lactic acid bacteria against several human pathogenic, enteric, putrefactive and spoilage type of organisms. Vakil and Shahani (1965) observed an in vitro inhibitory effect of \textit{L. acidophilus} against several Gram positive and some Gram negative organisms. Vincent \textit{et al.}, (1959) also claimed that antibiotic 'Lactocidin' from certain strains of intestinal \textit{L. acidophilus} had a broad antibacterial spectrum exhibiting bactericidal action against several Gram negative, Gram positive and acid-fast bacteria and molds.

Neri (1973) observed bacteriostatic effects of the substances produced by \textit{L. acidophilus} against \textit{B. subtilis}, \textit{Staph. aureus}, \textit{E. coli}, \textit{Salm. typhii}, \textit{Shigella dysentriae} and \textit{Brucella melitensis}.

Tomic-Karvoic (1963) observed bactericidal effects of the antibiotic substances of \textit{L. acidophilus} in the
whey of acidophilus milk against several pathogens. Prajapati et al., (1984) observed the antibacterial activity of *L. acidophilus*-LB1H3 against *E. coli* and concluded that the inhibition was attributed not only to the lactic acid but also some other antimicrobial compounds like hydrogen peroxide and other inhibitory substances.

Findings of Kim (1984) have shown that *L. acidophilus* grown in tomato juice produced an antimicrobial compound active against several test organisms. Alm (1985) reported that a daily consumption of more than 500 ml acidophilus milk shortened the duration of Salmonella carrier state in randomly selected human carriers. Prasad and Gandhi (1987) studied some factors affecting the production of antibacterial substances in *L. acidophilus* strain-R against fourteen different types of microorganisms.

The aforementioned antimicrobial effects are attributed to antibiotic/antibiotic-like products, acid(s), hydrogen peroxide and some unidentified/partially identified compounds by the lactic acid bacteria (Khedkar, 1988). It was observed that consumption of acidophilus milk and *L. casei* preparations resulted into
a lowering impact on Coliform counts and significant increases in faecal Lactobacilli in human subjects (Khedkar, 1988 and Khedkar et al., 2003).

Bifidus milk developed by Misra and Kuila (1992) using *Bif. bifidum*-NDRI exhibited antibacterial activity against *E. coli, B. cereus, Shi. dysenteriae* and *Staph. aureus* as determined by the agar diffusion technique. However, the inhibitory effects of organic acids and hydrogen peroxide had not been eliminated.

**2.12. Application of probiotics in newborns and children:**

Intestinal infections in newborn children are common and in developing countries diarrhoea is a prime cause of morbidity and mortality. In the United States, epidemiological estimates indicated that 21 to 37 million diarrheal disease episodes occurred in 16.5 million American children each year (Glass et al., 1991). Necrotizing enterocolitis is one devastating intestinal disorder that a preterm infant may face within a neonatal intensive care unit. Necrotizing enterocolitis is characterized by abdominal distension, bilious emesis, bloody stools, lethargy, apnea (Caplan and Jilliug 2000).

The disease progresses through an inflammatory
cascade with septic shock and intestinal necrosis. Necrotizing enterocolitis has been reported to occur in 10 to 25% of preterm infants (1,500 g in weight) admitted to the neonatal intensive care unit, and it may involve approximately one bacterial colonization or infection of the intestine by pathogens such as Clostridium, Escherichia, Klebsiella, Salmonella, Shigella, Campylobacter, Pseudomonas, Streptococcus, Enterococcus, *Staphylococcus aureus* and coagulase-negative staphylococci increases the risk of necrotizing enterocolitis. If nonpathogens, such as lactobacilli and bifidobacteria, colonize the intestine or if breast milk rather than formula is used, the incidence of necrotizing enterocolitis has been reported to fall (Lucas and Cole, 1990).

At the time of this finding in 1990, these authors estimated that in British neonatal units, exclusive formula feeding could account for approximately 500 extra cases of necrotizing enterocolitis and 100 deaths each year. No recent comparison is available, but changes in infant formulas have been made over the past 13 years, so conclusions drawn from that time may or may not be relevant today.
2.12.1. Low birth weight infants:

Low-birth-weight premature infants delivered by caesarian section are often ill equipped for life outside the womb. They require intensive care, and for those who are breast fed, the feeding usually only begins several days after the infants are exposed to a plethora of microbes, many of which have pathogenic potential. This indicates that the normal process by which organisms such as lactobacilli are ingested via vaginal birth and propagated by the mother's milk do not take place. As a result, this may allow pathogens to establish within the premature intestine. Furthermore, infants given antibiotics at birth retain an abnormal micro biota 4 weeks later; such is the dramatic impact of these agents (Gewolb et al., 1999).

The intestinal microbiota in low-birth-weight premature infants can be dominated by many pathogens such as Enterococcus faecalis, E. coli, Staph. epidermidis, Enterobacter cloacae, Klebsiella pneumoniae, and Staph. haemolyticus (Gewolb et al., 1999 and Mackie et al., 1999). In particular, Clostridium peifringens has been isolated from 40% of babies with necrotizing enterocolitis, compared with 13% of controls.
(P=0.03). However, in premature infants given breast milk, lactobacilli and bifidobacteria are present in a more diverse microbiota. For example, in a study of the enteric microbiota of 25 babies with necrotizing enterocolitis compared to 23 matched controls, lactobacilli were less common in the necrotizing enterocolitis babies (12% versus 48%, P=0.006) (Blakey et al., 1985). These findings suggested a correlation between the reduction of lactobacilli and the increased risk of necrotizing enterocolitis.

Other studies indicated that bifidobacteria not only colonized the gut of animals, possibly helping to exclude pathogens; they also reduced endotoxemia and appeared to modulate the inflammatory cascade (Caplan and Jiliug 2000). Perhaps the most impressive indication that probiotics could benefit newborns comes from a human trial with 2.5X10⁸ live L. acidophilus and 2.5X10⁸ live Bif. infantis in 1,237 neonates in Colombia. Compared with 1,282 hospitalized patients seen during the previous year, treatment with these strains resulted in a 60% reduction in necrotizing enterocolitis and overall mortality (Hoyos, 1999). Although historical comparisons are not an ideal clinical trial design, the
results nevertheless are striking and warrant consideration. A subsequent study involved newborn infants with a gestational age of <33 weeks or birth weight of <1,500 g and a standard milk feed supplemented with *Lactobacillus* sp. strain GG in a dose of $6 \times 10^9$ CFU once a day until discharge (47 days) (Dani *et al.*, 2002). The study found a reduced rate of necrotizing enterocolitis compared to placebo (1.4% versus 2.7%) but was not statistically significant, suggesting that either the GG strain is not as good as the *L. acidophilus*- *B. infantis* combination, milk is not an effective delivery system, or probiotics are not as effective as earlier thought (Hoyos, 1999). A further study of enteral feeding of premature infants with *Lactobacillus* sp. strain GG showed that the organism could be recovered from the stool and was thus delivered and survived passage, even though it did not appear to confer any detectable benefits (Millar *et al.*, 1993).

Failure of the GG strain to prevent necrotizing enterocolitis does not necessarily indicate a lack of benefit to newborns. A double-blind, randomized, placebo-controlled trial involving 132 participants over a 2-year period showed that daily feeding of two
capsules containing $10^{10}$ CFU of *Lactobacillus* sp. strain GG to pregnant mothers who had at least one first-degree relative (or partner) with atopic eczema, allergic rhinitis, or asthma and after birth to the mother and to the babies for 6 months significantly reduced the incidence of allergic atopic dermatitis (15 of 64 (23%) versus 31 of 68 (46%), $P = 0.0008$) (Kalliomaki *et al*., 2001). This implies a functional modulation of immunity rather than a specific antipathogen reaction in the gut. This effect has now been shown to remain at 4-year follow up (Kalliomaki *et al*., 2003).

The immune response within the gastrointestinal tract is a fine balance between the release of proinflammatory (e.g., interleukin-1, -6, and -8 and tumor necrosis factor) and antiinflammatory (e.g., interleukin-1RA, -4, and -10) cytokines. In a review on mucosal immunity starting at birth, Walker (2000) reported a correlation between a normal gut microbiota and protection against various infections. This is an important observation because it supports the concept of early intestinal colonization with organisms such as lactobacilli and bifidobacteria and possibly subsequent protection from enterocolitis and other diseases.
It is estimated that every 15 s a child dies from diarrheal disease somewhere in the world. In a study in 204 undernourished, 6- to 24-month old children in Peru, once-daily intake of \textit{L. rhamnosus-GG} 6 days a week for 15 months led to significantly fewer episodes of diarrhea (5.21 versus 6.02 episodes of diarrhea per child per year in the placebo group; P=0.028) (Oberhelman \textit{et al.}, 1999). However, this type of study is difficult to verify because there is little control over the organisms to which the children are exposed and the compliance in taking the treatment. Probiotics provide a safe and potentially beneficial remedy, especially when delivered in milk, which provides the child with nutrition and a means to overcome adverse effects of fluid loss. Current WHO recommendations state that clinical management of acute diarrhea should include replacement of fluid and electrolytes losses along with nutritional support (WHO, 1995).

The strongest evidence of a beneficial effect of probiotics has been established with some of the strains of Lactobacilli for prevention and treatment (Gnarino \textit{et al.}, 1997; Guandalini \textit{et al.}, 2000 and Szajewska \textit{et al.}, 2001) of acute diarrhea mainly caused by rotaviruses in
children. The study designs cited are similar, randomized, double blinded, and placebo controlled. The statistically significant reduction in the duration of diarrhea is consistent and quite convincing, especially for the GG strain used in several of the trials. Note also that unlike many pharmaceutical treatments, there were no significant side effects reported. In a European study, faster hospital discharge was achieved in addition to improvement in clinical outcome (Gnarino et al., 1997). Two hundred ninety-one children 1 to 3 months of age were randomly allocated to receive oral rehydration solution plus placebo or 10^10 L. rhamnosus GG. After rehydration in the first 4 to 6 h, patients were offered their usual feedings plus free access to the same solution until diarrhea stopped. Duration of diarrhea was reduced from an average of 3 days to 2.4 days (P = 0.03). In a randomized, placebo-controlled study of 40 patients between 6 and 36 months of age hospitalized with acute diarrhea (75% rotavirus), treatment with high doses of L. reuteri (assumed to be strain SD2222) (10^{10} to 10^{11} CFU) for up to 5 days resulted in reduction in duration of watery stools (1.6 versus 2.9 days in the placebo group) (P=0.07) (Shornikova et al., 1997). In a
second prospective, randomized, placebo-controlled trial of children between 6 and 36 months of age admitted for rotavirus-associated diarrhea, three groups received either $10^{10}$ or $10^7$ CFU of *L. reuteri* SD2222 or a matching placebo once a day for up to 5 days. The outcome supported the earlier findings, with a mean duration of watery diarrhea being optimal for patients given the highest dose of lactobacilli (1.5 days versus 1.9 days for the lower dose versus 2.5 days for the placebo group) (Shornikova *et al.*, 1997a). In summary, it is believed that there is sufficient evidence to recommend use of probiotic strains in capsule or milk form, to treat acute diarrhea in children, in combination with standard oral rehydration.

2.13. Applications of probiotics to combat GIT infections:

Probiotics have been shown to be useful in the treatment of a variety of gastrointestinal disorders. A number of these disorders have a significant inflammatory component in the small and large intestine and there is a growing body of research to suggest that probiotic bacteria may be useful particularly in many of these paediatric gastrointestinal conditions. *L. casei*
ssp. *rhamnosus* (LGG), *L. reuteri*, *L. plantarum*, *Bif. bifidus* with *Str. salivarius* ssp. *thermophilus* and *Saccharomyces boulardii* have all been extensively studied. Probiotics can reduce the duration and severity of rotaviral enteritis, as well as decrease the risk of antibiotic associated diarrhoea in children and *Clostridium difficile* diarrhoea in adults. Prevention of viral diarrhoea in day care settings as well as traveller's diarrhoea has been demonstrated with some probiotics. Small bowel bacterial overgrowth conditions may respond to probiotic use.

**2.14. Possible mode of action of probiotics:**

Several potential mechanisms have been proposed for how probiotics reduce the duration of rotavirus diarrhea, but none have been proven and each theory has flaws. The first is competitive blockage of receptor sites (Bernet *et al.*, 1994) in which probiotics bind to receptors, thereby preventing adhesion and invasion of the virus. This concept might be plausible if there was evidence for specific receptor competition. In most cases, by the time a probiotic is ingested, the patient will already have had diarrhea for possibly 12 h. By this time, the virus has infected mature enterocytes in the
mid- and upper region of the small intestinal villi. The virus and/or its enterotoxin, NSP4 will then have inhibited fluid and electrolyte transport, thereby lowering fluid and glucose absorption. The toxin could have then potentially activated secretory reflexes, causing loss of fluids from secretory epithelia, resulting in diarrhea (Lundgren and Svensson, 2001).

At the best, subsequent competitive exclusion of viruses would only be effective for attachment of progeny, and it is not known whether such inhibition would reduce diarrhea. If probiotic organisms somehow competed with the toxin or peptides released from villous endocrine cells, it is feasible that the cascade that leads to diarrhoea could be prevented. The second potential mechanism may be that the immune response is enhanced by probiotics, leading to the observed clinical effect (Kalia et al., 1992). This is supported by the protective effect which local Immunoglobulin A (IgA) antibodies appear to confer against rotavirus (Ward et al., 1996). However, a problem with this theory is given that diarrhoea appears to cease within 1 to 3 days in patients who would otherwise suffer for 4 to 6 days; the
prbiotics would need to trigger the antibody response rapidly so that it interfered with further viral activity.

Animal studies do indicate that secretory IgA can be triggered by probiotic ingestion (Reid et al., 2002), but the rate was not determined, nor was the influence on cessation of fluid loss across the secretory cell membranes. Modification of the cytokine profile to one that enhances anti-inflammatory cytokines (Christensen et al., 2002) or attenuation of the virus's and/or toxin's effect on the enteric nervous system might provide rapid cessation of epithelial secretion and diarrhea. Alternatively, stimulation of T cells to produce gamma interferon, leading to potential inhibition of chloride secretion, might also inhibit diarrhea. One aspect of the immunity theory that needs to be clarified is why lactobacilli, which we assume are present in the child intestine, appear unable to prevent infection; yet those administered orally thereafter help to clear the diarrhoea.
A third mechanism could involve a signal(s) from probiotics to the host that down regulates the secretory and motility defenses designed to remove perceived noxious substances. Glycosylated intestinal mucins inhibit rotaviruses (Yolken et al., 1994), and MUC2 and MUC3 mRNA expression is increased in response to probiotics signaling, protecting cells against pathogenic bacterial adhesion (Mack et al., 1999). However, direct host cell signaling between probiotic organism and secretory cells is not investigated. Attachment of the virus causes cytokine prostaglandin and nitric oxide release from the enterocytes, both of which could affect motility. Possibility exists that lactobacilli could alter this release (Xu and Verstraete, 2001).

The intestinal host defense mechanisms comprise complex systems involving the innate and adaptive immune responses, and protective effects of the indigenous microbiota. The commensal microorganisms colonizing the intestinal mucosa provide a barrier effect against pathogens by using a variety of mechanisms, such as occupation of niches, competition for nutrients and production of antimicrobials. It is also established that the probiotic organisms can modulate the
homeostasis of the host's defense mechanisms, both innate and adaptive immune functions (Vaughan et al., 1999).

A final theory is that the probiotics produce substances that inactivate the viral particles. This has been shown in vitro (Cadieux et al., 2002), with supernatants from *L. rhamnosus* GR-1 and *L. fermentum* RC-14 inactivating 109 particles of the double stranded DNA adenovirus and the negative-stranded RNA vesicular stomatitis virus within 10 min. The effect was likely due to acid, but more specific antiviral properties have not been ruled out. Whether or not viral killing activity can inhibit diarrhea remains to be confirmed. More detailed investigation is needed to understand how probiotic strains reduce the duration of diarrhoea in conjunction with rehydration therapy. Such studies could lead to a better understanding of the dynamics within the intestinal microbiota that is being disrupted and depleted by rapid fecal loss. In doing so, new interventional therapies should be generated to quickly and effectively trigger the cessation of not only rotavirus illness but also other gastrointestinal infections that debilitate patients for 2 to 3 days.
2.15. Modulating the GIT microbiota through intake of probiotics:

In the human gastrointestinal tract, there exists variability in bacterial numbers and populations between the stomach, small intestine and colon. The total bacterial count in gastric contents is usually below 10^3 per g with numbers in the small intestine ranging from about 10^4 per ml of contents to about 10^6-10^7 at the terminal ileum (Gorbach et al., 1967). In comparison to other regions of the gastrointestinal tract, the human large intestine is a complex, heavily populated and diverse microbial ecosystem. Bacterial numbers in the human large intestine are in the range of 10^{11}-10^{12} for every gram of the gut contents (Cummings and Macfarlane, 1991).

The colonic microflora is capable of responding to anatomical and physicochemical variations that are present. The right or proximal colon is characterised by a high substrate availability (due to dietary input), a pH of around 5.5-6.0 (from acids produced during microbial fermentation) and a more rapid transit than the distal region. The left or distal area of colon has a lower concentration of available substrate, the pH is
approximately 6.5-7.0 and bacteria grow more slowly. The proximal region tends to be a more saccharolytic environment than the distal gut, the latter having higher bacterial proteolysis. Several hundred different species of bacteria are thought to be present in the large intestine. Gram-negative rods belonging to the Bacteroides fragilis group are the numerically predominant culturable bacteria in the colon. The other main groups consist of different (Gram positive) rods and cocci, such as bifidobacteria, clostridia, peptococci, streptococci, eubacteria, lactobacilli, peptostreptococci, ruminococci, enterococci, coliforms, methanogens, dissimilatory sulphate-reducing bacteria and acetogens. The flora includes saccharolytic organisms, proteolytic species and bacteria that can metabolise gases. Despite the huge diversity of bacteria thought to be present in the large gut (about 500 described species), it is certain that the vast majority has not been hitherto identified (Fooks et al., 1999).

One of the most important health benefits of consuming the probiotic organisms is their ability to adhere to the intestinal mucosa. As such they can resist peristalsis and occupy a niche at the expense of harmful
organisms. The probiotic applications to the human gut are already widespread, and evidence is mounting that these organisms have a beneficial effect on the host. It is now well established that the probiotic organisms can establish in the gastrointestinal tract and inhibit the adhesion and growth of enteropathogens.

2.16. Effect of feeding probiotics on GIT microflora:

In the human gastrointestinal tract, there exists variability in bacterial numbers and populations between the stomach, small intestine and colon. The total bacterial count in gastric contents is usually below $10^3$ per g with numbers in the small intestine ranging from about $10^4$ per ml of contents to about $10^6$-$10^7$ at the terminal ileum (Gorbach et al., 1967). In comparison to other regions of the gastrointestinal tract, the human large intestine is a complex, heavily populated and diverse microbial ecosystem. Bacterial numbers in the human large intestine are in the range of $10^{11}$-$10^{12}$ for every gram of the gut contents (Cummings and Macfarlane, 1991). The colonic microflora is capable of responding to anatomical and physicochemical variations that are present. The right or proximal colon is characterised by a high substrate availability (due to
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the vast majority has not been hitherto identified (Fooks et al., 1999).

2.17. Increasing the numbers of beneficial microbes in the GIT:

One of the most important health benefits of consuming the probiotic organisms is their ability to adhere to the intestinal mucosa. As such they can resist peristalsis and occupy a niche at the expense of harmful organisms. The probiotic applications to the human gut are already widespread, and evidence is mounting that these organisms have a beneficial effect on the host. It is now well established that the probiotic organisms can establish in the gastrointestinal tract and inhibit the adhesion and growth of enteropathogens. Table 2.3 delineates the effect of feeding probiotic preparations on the human gut microbiota.
TABLE 2.3: EFFECT OF FEEDING PROBIOTIC PREPARATIONS ON HUMAN GUT MICROBIOTA

<table>
<thead>
<tr>
<th>Type of probiotic organisms</th>
<th>Effect on gut microbiota</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. rhamnosus</em> GG (ATCC 53103)</td>
<td>Attachment of probiotic organism to CaCo-2 intestinal cell line and in vivo to human colonic mucosa</td>
<td>Alander et al., 1999</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> GG</td>
<td>Increased the number of faecal bifidobacteria and lactobacilli. Concomitant decrease in clostridia counts.</td>
<td>Benno et al., 1996</td>
</tr>
<tr>
<td><em>L. plantarum</em> (VTTE-79098)</td>
<td>Reduction in enterobacteriaceae counts of 4 log cycles, Clostridia 1 log cycle and slight decreases in enterococci counts in a SHIME reactor</td>
<td>Alander et al., 1999</td>
</tr>
<tr>
<td><em>L. paracasei</em> ssp. paracasei (VTTE-94506)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. paracasei</em> ssp. paracasei (VTTE-94510)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium sp. (VTTE-94508)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. casei</em> shirota</td>
<td>Balancing of intestinal microflora</td>
<td>Aso and Akazan, 1992</td>
</tr>
<tr>
<td><em>Bif. Bifidum</em></td>
<td>Balancing of intestinal microflora</td>
<td>Marteau et al., 1990</td>
</tr>
<tr>
<td><em>L. acidophilus</em>-LBKV3</td>
<td>Highly significant increases in faecal LAB counts and concomitant decreases in harmful types.</td>
<td>Khedkar et al., 2003</td>
</tr>
<tr>
<td><em>L. acidophilus</em>-LBKV3 supplimented with Prop. freundenrichii ssp. Shermanii</td>
<td>Supplementation of probiotic strain of <em>L. acidophilus</em> caused significant increases in <em>in vivo</em> antimicrobial activity.</td>
<td>Khedkar et al., 2004</td>
</tr>
</tbody>
</table>
2.18. Suppressing numbers of potentially harmful microbes:

The artificial manipulation of human intestinal microbiota by consumption of large numbers of probiotic microorganisms mimics the natural, be it artificially induced, overgrowth of abundantly lactic acid-producing organisms in short small bowel syndrome patients (Bongaerts et al., 1997) in small intestine available sugars are quickly fermented to lactic acid and/or ethanol. L-lactate is easily metabolised in all humans, in contrast to D-lactate and ethanol, which are normally metabolised only in older children and adults. Production of all kinds of the low-molecular toxic metabolites and antigenic macromolecules by various intestinal, more or less pathogenic microbes and the effects of endotoxins may be strongly reduced.

The intestinal growth of all other types of non intestinal pathogens is strongly inhibited by abundant probiotic fermentation in the small intestine. Reduction of viral infectivity was attributed to ethanol or acid-mediated denaturation of viral envelope proteins. In addition to lactic acids the intestinal bacteriocins,
nisin, antibiotics and some unidentified compounds synthesized by probiotic organisms confer a final growth-inhibiting effect (Bongaerts and Severijnen, 2001).

2.19. Incorporation of probiotics in beverages:

The utilization of pomegranate juice at @ 0 (T1), 10 (T2), 15 (T3) and 20% (T4) with 10% sugar was mixed in chakka whey for manufacture of beverage. The different levels of pomegranate juice had a definite effect on improving the sensory quality of the beverage. The beverage prepared by utilizing chakka whey with 15% pomegranate juice (T3), had secured the highest sensory score (8.81) and ranked as most acceptable product followed by T2 with 8.65 points sensory score. The percentage of protein total sugar, ash and acidity (% LA) of the product increased with increase in the levels of pomegranate juice (Babar et al., 1971).

In recent years there has been much interest in low calorie food products and probiotics. Incorporation of probiotics in tropical fruit juices can result in excellent therapeutic value and effective calorie reduction.
A process was standardized to develop whey beverages in combinations with and without skim milk using selected strains of *L. acidophilus*-NCDC-15, *L. casei*-NCDC-12 and *L. casei*-RTS, which have antagonistic properties and bile salt tolerance. *L. acidophilus*-NCDC-15 in combination with *L. casei*-NCDC-12 produced a highly acceptable beverage which had antagonistic properties against food borne pathogens (*E. coli, Kleb. pneumoniae, Sal. typhi* and *Staph. aureus*) and showed maximum tolerance at pH 4.0 and in presence of 0.5% bile salt. The growth characteristics of selected strain were studied in 50:50 and 100:00 blends of whey and skim milk at 37±1°C following 16 h of incubation. *L. acidophilus*-NCDC-15 in combination with *L. casei*-NCDC-12 strains produced highly rated acceptable beverage as determined by a sensory panel and also was best in terms of lowest pH, maximum titratable acidity, rate of acid production, percent soluble nitrogen, and acetaldehyde production (Tripathi and Jha, 2004).

In recent years there has been much interest in low calorie food products and probiotics. Incorporation of *L. acidophilus* and *Bifidobacterium* in fermented
beverages can result in a milk product with excellent therapeutic value and effective calorie reduction. The objective was to study the effect of whey, sugar and fructo oligosaccharides on the lactic acid bacteria population of twelve formulations of fermented beverages. Populations were determined from \textit{Str. thermophilus}, \textit{L. delbrueckii} subsp. \textit{bulgaricus}, \textit{Bif. bifidum} and \textit{L. acidophilus}. The largest populations of probiotic microorganisms were observed in beverages with lower acidity and higher solids content, besides having a predominance of \textit{Str. thermophilus} on the other microorganisms. Samples attended the Brazilian legislation in force, regardless of the formulations, the presence of at least $10^6$ cfu/ml of lactic acid bacteria. The dairy beverages formulated probiotic may be considered by the high scores of \textit{Bifidobacterium} spp. and \textit{L. acidophilus}. (Karime and Ana Lúcia, 2005).

In this category of products big attention has been paid to development of probiotic whey beverages, since beneficiary effects of probiotic strains on human health like lowering cholesterol level in blood, improving lactose metabolism, lowering blood pressure, anticancerogenic properties and immune system
stimulation are known for a long period of time (Shah, 2007).

One of the most important factors is the chosen probiotic strain since it determines the unique flavor and texture of the end product. In the past few years many studies regarding fermentations with probiotic strains *L. reuteri* and *Bif. bifidum* have been made whereby Mendoza *et al.*, (2007) managed to produce an acceptable probiotic whey beverage with addition of sugar and pectins.

The study was conducted on survival and growth of probiotic strains *L. acidophilus-La-5*, *Bif. bifidum-Bb-12* and *L. casei-Lc-1* in reconstituted whey for 28 days under cold storage. All strains have shown good survival during storage time of fermented beverages. The beverage fermented by probiotic strain Bb-12 obtained lower sensory score than the other two beverages fermented by strains La-5 and Lc-1 (Jelićić *et al.*, 2008).

Whey beverage was prepared by utilizing *L. rhamnosus*-NCDO 243, *Bif. bifidum*-NCDO 2715 and *Prop. freudenreichii* subsp. *shermanii*-MTCC 1371 in order to make a fermented probiotic healthy drink. The
product made with 4% mixed culture (1:1:1) inoculated (initial counts: Lactobacilli 6.2X10^7 cfu/ml, Bifidobacteria 5.4X10^7 cfu/ml, and Propionibacteria 3.9X10^7 cfu/ml) in deproteinized whey (4.6% lactose, 0.62% ash, 0.48% fat and 0.5% protein) adjusted to pH 6.4 and incubated at 37°C for 8 h has a good technological and dietetic criteria required for a probiotic product. Total bacterial count, lactobacilli count, bifidobacteria count, propionibacteria count, titratable acidity, β-D galactosidase activity, concentration of lactic acid and sensory properties were monitored during storage period. The whey beverage fermented for 8 h and prepared with 4% inoculum of mixed culture (1:1:1) met the probiotic criteria by maintaining each type of bacterial population at counts greater than 10^8 cfu/ml up to 10 days of storage period. The titratable acidity as well as sensory properties did not change appreciably during first 7 days of storage. At the end of 15 days of storage, slight acidification was detected, although the beverage still retained an acceptable flavour (Maity et al., 2008).

A soft beverage was prepared from Paneer whey (65%) with guava pulp (25%) and sugar (10%) which was
pasteurized at different temperatures and timings i.e. 60°C-70°C for 15-35 minutes. The prepared beverages were evaluated for their physico-chemical properties and organoleptic qualities every 15 days till 45 days. Effect of different temperatures, timings and storage periods on the mean sensory sources of whey-guava beverage was significant. It was also observed that there were significant changes in total sugars, reducing sugars, non reducing sugars and vitamin C during the storage period. However, whey-guava beverages pasteurized at 70°C for 35 minutes was found best in terms of sensory quality after 45 days. The pH, acidity, protein, total sugars and reducing sugars found to be higher than that of the other samples. (Divya and Archana Kumari, 2009).

The development and storage of whey-based banana herbal (WBBH) beverage with the incorporation of *Mentha arvensis* extract (0-4%). The amount of banana juice and sugar were fixed at 10 ml and 8 g, respectively per 100 ml of the beverage. Whey quantity varied from 72 to 84 ml for each 10 ml of the beverage depending upon the concentration of mentha extract.
The storability of the beverage was studied at 7±1°C for 20 days. The organoleptic scores and overall acceptability of the beverage improved with increase in mentha extract from 0-2%. Addition of 3 and 4% mentha extract decreased the beverage quality as beverage scored lower organoleptic score. Acidity and reducing sugar content increased while pH decreased during storage. The overall acceptability of the beverage was desirable up to 15 days of storage at refrigeration temperature. Beverage prepared from banana juice and whey in combination with edible extract of herbal medicinal plants like *M. arvensis* will not have only excellent nutritional properties but will also posses therapeutic, prophylactic, antibacterial and organoleptic properties (Ritika Yadav et al., 2010).

A study aimed at optimization of the conditions of *L. casei*-NRRL B-442 cultivation in cashew apple juice and to determine the proper inoculum amount and fermentation time was conducted. The survivability of *L. casei* in cashew apple juice during refrigerated storage (4°C) for 42 days was investigated. The optimum conditions for probiotic cashew apple juice production were initial pH 6.4, fermentation temperature of 30°C,
inoculation level of 7.48 Log cfu/ml (L. casei) and 16 h of fermentation process. It was observed that the L. casei grew during the refrigerated storage. Viable cell counts were higher than 8 Log cfu/ml throughout the storage total color change increased and the values of redness reduced along the fermentation and refrigerated storage periods. The fermented juice with L. casei is a good and healthy alternative functional food containing probiotics. Cashew apple juice showed to be as efficient as dairy products for L. casei growth (Lúcia F. Pereira, 2010).

2.20. Improving viability of probiotics in various products:

Despite the importance of viability of these beneficial microorganisms, many surveys have shown unsatisfactory counts of live bacteria in market samples of fermented products (Hull et al., 1984; Shah et al., 1995). Most Australian yoghurts did not contain L. acidophilus at viable populations greater than 10^6 cfu/g, causing doubts about their ability to provide any probiotic benefits to consumers supplemented lactobacilli, the numbers of the these bacteria had decreased by 92.6 percent during 51 days of storage at
7°C, the product still contained populations of $1 \times 10^6$ cfu/g at the end of the storage period, and was likely to be of some dietetic and therapeutic value. Yet, the loss in viability of the probiotic bacteria was significant (Medina and Jordano 1994) and could not be ignored.

In recent years, many researchers have attempted to improve the viability of probiotics in various products. A two-step fermentation increased the counts of *L. acidophilus-2409* and *Bif. longum-1941* in yoghurt by five times. Also, their viability during 6 weeks of storage at 4°C was satisfactory. Processed milk was inoculated with *L. acidophilus 2409* and *Bif. longum-1941* @2% each and incubated at 42°C for 2 h. This was followed by inoculation with *L. bulgaricus-1255* and *Str. salivaricus* ssp. *thermophilus-2010* @ 0.5% each. The process increased the fermentation time by 2 h. Neutralization of yoghurt mix before fermentation to a pH of 6.8 to 6.9 using calcium hydroxide Ca(OH)$_2$, increased the counts of probiotic cultures by 4 to 6 times. This method increased the process time by 20 to 60 minutes. Probiotic yoghurt prepared by both methods as well as control samples showed similar levels of acetaldehyde. The counts of the probiotic bacteria
declined in all the products during refrigerated storage (Lankaputhra and Shah, 1997).

Viable counts of probiotic bacteria (L. acidophilus-2409; Bif. longum-1941; Bif. infantis-1912; Bif. bifidum-1900, 1901 and Bif. pseudolongum-20099) were two log cycles higher, in yoghurt made with ruptured yoghurt bacteria and whole cells of probiotic bacteria, and their viability after 6 weeks of storage at 4°C remained above the recommended level of $10^6$ cfu/g. This was possibly due to the higher levels of β-galactosidase released as a result of rupture of yoghurt bacterial cells. Yoghurt thus prepared, contained lower levels of $H_2O_2$ and similar levels of acetaldehyde, as compared to conventional yoghurt (Shah and Lankaputra, 1997). This approach has been suggested as a method for improving the viability of probiotic bacteria without compromising the flavour of the product.

Ascorbic acid (Vitamin C) can act on oxygen scavenger and is permitted in fruit juices and other products as a food additive. Since milk and milk products supply only 10-15% of the daily requirement of Vitamin C, fortification of yoghurt with ascorbic acid...
would increase its nutritive value. Supplementation with ascorbic acid at the rate of 250 mg/kg marginally improved the viability of Lactobacillus species in probiotic yoghurt during the initial 15 to 20 days of storage at 4°C, after which the dissolved oxygen content and redox potential rapidly approached unaffected, while the multiplication of *Str. salivaricus ssp. thermophilus* was adversely affected (Dave and Shah, 1997c).

2.21. Whey as a carrier medium for probiotics:

Lactobacilli and bifidobacteria, which are the most prominent candidature organisms as probiotics must have the ability to survive the harsh conditions in the gut if they are to be used as dietary adjuncts in fermented foods. Proteolytic enzyme i.e. pepsin can hydrolyze the proteins of the outer layer of bacterial cells in acidic condition (optimum pH 1.0-2.5) in the stomach. Moreover, bacteria have cell membranes consisting of lipids and fatty acids that are very susceptible to destruction by bile salts.

However, the survival during passage through the GI tract is influenced by the nature of food carrier used
for the delivery of probiotics. Whey can protect the cell from reaching the death by increasing the overall pH and inhibiting digestive protease activity (Goyal and Gandhi, 2008). In a study, *L. rhamnosus* strain VTT E-97800 (E800) or Lc705 (the latter in combination with *Prop. freudenreichii ssp. shermanii* JS) were separately administered to healthy adult volunteers in a whey-based fruit juice. Both *L. rhamnosus* strains were recovered in high numbers in faecal samples during the consumption period. The results indicated that *L. rhamnosus* strains E800 and Lc705 had good survival ability in the GI-tract when administered in a whey-based fruit juice matrix.

Considering the potential of whey, based on its nutritional value, the aim of the study conducted by Ida Drgali (2005) was to define the growth and survival of probiotic bacteria in whey and the influence of prebiotic inulin addition on it, for possible production of a nutritive highly valuable whey drink. To get the same experimental conditions, the fermentation was conducted in reconstituted whey with approximately 6% of total solids. The reconstituted whey was pasteurized and inoculated with three types of commercial probiotic
culture: La-5, Bb-12 and Lc-01. Inoculated samples were fermented at 37°C for 24 h and sensory evaluated. Beverages with the highest sensory scores (after 18 h of fermentation) were cool stored to determine stability. After 28 d of cool storage the bacterial count was higher than 10^7 cfu/ml and spoilage was not detected in any sample. Inulin addition had an almost negligible effect on bacterial count during fermentation and cool storage.

Yoghurt is a potential source of probiotic lactobacilli. In the study conducted by Hoque et al., (2010) Lactobacillus spp. were isolated from two regional yoghurts in Bangladesh, which were identified on the basis of their colony morphologies and some biochemical tests. It was observed that isolated Lactobacillus spp. were resistance to inhibitory substances like phenol (0.4%), NaCl (1-9%) and bile acid (0.05-0.3%). Additionally, good growths were observed in the presence of 1% NaCl and 0.3% bile acid. The isolated Lactobacillus spp. did show good survival abilities in acidic (pH 2.5) and alkaline (pH 8.5) conditions, while, their maximum growth was observed at pH 5.0 for lactobacilli isolated from Bogra yoghurt.
and at pH 6.5 for lactobacilli isolated from yoghurt of Khulna region of Bangladesh. Isolated lactobacilli were able to produce organic acid in skim milk which was determined by titrimetric method. The Lactobacillus spp. also did show good survival abilities in simulated gastric juice at pH 2.22 and pH 6.6 (Control). Their susceptibility to selected nine antibiotics was determined in terms of minimum inhibition concentration (MIC). The MICs results showed that, Lactobacillus spp. isolated from Bogra yoghurt were sensitive to amoxicillin, moderately sensitive to gentamycin, clindamycin, azithromycin and resistant to kanamycin, nalidixic acid, metronidazol, cefradine and tetracyclin. On the other hand, Lactobacillus spp. isolated from yoghurt of Khulna region were sensitive to gentamicin, clindamicin and resistant to amoxicillin, tetraciclin, kanamicin, nalidixic acid, metronidazol, azithromycin and cefradine. Most of the results from this investigation showed that, there were variations in probiotic properties of the isolated Lactobacillus spp. from different regions.
2.22. Tropical fruit juices as delivery medium for probiotics:

The effectiveness as protective culture of the probiotic *Lactobacillus rhamonosus* GG (*L. rham. GG*) against *Salmonella* and *Listeria monocytogenes* on minimally-processed apples throughout storage as well as its effect on apple quality and natural microflora was evaluated. Survival to subsequent exposure to gastric stress was also reported. Apples were cut into wedges and dipped in a solution containing *Salmonella* and *L. monocytogenes* (10^5 cfu/ml) and/or *L. rham. GG* (10^8 cfu/ml). Apple wedges were packed and stored at 5 and 10 °C. Periodically, microbial population, bacterial survival to gastric stress and quality of apple wedges were evaluated. Although *Salmonella* was not affected by co-inoculation with *L. rham. GG*, *L. monocytogenes* population was 1-log units lower in the presence of *L. rham. GG*. *L. rham. GG* population maintained over recommended levels for probiotic action (10^6 cfu/g) along storage, however, viable cells after gastric stress were only above this level during the first 14 days. Pathogen survival after gastric stress was <1% after 7 days at 5 °C. Moreover, apple wedges quality was not affected by *L. rham. GG* addition. Thus, *L. rham. GG*...
could be a suitable probiotic for minimally-processed apples capable to reduce *L. monocytogenes* growth; nevertheless shelf life should not be higher to 14 days to guarantee the probiotic effect (Isabel, *et al*., 2011).

The aim of the work conducted by Sawaminee and Dimitris (2011) was to study the survival of *Lactobacillus plantarum* NCIMB 8826 in model solutions and develop a mathematical model describing its dependence on pH, citric acid and ascorbic acid. A Central Composite Design (CCD) was developed studying each of the three factors at five levels within the following ranges, i.e., pH (3.0–4.2), citric acid (6–40 g/L), and ascorbic acid (100–1000 mg/L). In total, 17 experimental runs were carried out. The initial cell concentration in the model solutions was approximately $1 \times 10^8$ cfu/ml; the solutions were stored at 4°C for 6 weeks. Analysis of variance (ANOVA) of the stepwise regression demonstrated that a second order polynomial model fits well the data. The results demonstrated that high pH and citric acid concentration enhanced cell survival; one the other hand, ascorbic acid did not have an effect. Cell survival during storage was also investigated in various types of juices, including orange,
grapefruit, blackcurrant, pineapple, pomegranate, cranberry and lemon juice. The model predicted well the cell survival in orange, blackcurrant and pineapple, however it failed to predict cell survival in grapefruit and pomegranate, indicating the influence of additional factors, besides pH and citric acid, on cell survival. Very good cell survival (less than 0.4 log decrease) was observed after 6 weeks of storage in orange, blackcurrant and pineapple juice, all of which had a pH of about 3.8. Cell survival in cranberry and pomegranate decreased very quickly, whereas in the case of lemon juice, the cell concentration decreased approximately 1.1 logs after 6 weeks of storage, albeit the fact that lemon juice had the lowest pH (pH ~ 2.5) among all the juices tested. Taking into account the results from the compositional analysis of the juices and the model, it was deduced that in certain juices, other compounds seemed to protect the cells during storage; these were likely to be proteins and dietary fibre. In contrast, in certain juices, such as pomegranate, cell survival was much lower than expected; this could be due to the presence of antimicrobial compounds, such as phenolic compounds.
A study was conducted by Saarela, et al. (2011*) to investigate the acid tolerance of mutants of probiotics. The potential of UV mutagenesis combined with a specific selection step to generate more acid-resistant *Bif. animalis* subsp. *lactis* Bb-12 strains with improved viability in low pH food matrices was investigated in this study. A total of 144 Bb-12 UV-mutants were initially characterised. After prolonged storage in apple juice (pH 3.5) and various phenol- and genotypic tests (acid and bile tolerance, substrate utilisation, antibiotic susceptibility, aerotolerance, RAPD) two mutants (2.20 and 2.56) were chosen for further studies including cell surface morphology, stability of various traits after repeated inoculations, and performance in a fermentar and during down-stream processing. 2.20 and 2.56 showed over two Log-values better viability in pH 3.5 juice compared to Bb-12. Alterations in cell surface structures of 2.20 and 2.56 were detected with AFM, whereas other studied traits remained unchanged. In conclusion, UV mutagenesis and subsequent incubation in acidic medium enabled improving the stability of *B. animalis* subsp. *lactis* in low pH juice. Acid tolerance testing (HCl, pH 2.5, 2 h) results did not predict long-
term stability of the strains in acidic food matrix (apple juice pH 3.5).

Bifidobacterial food applications are limited since bifidobacteria are sensitive to e.g. acidic conditions prevalent in many food matrices. The aim of the present study was to investigate whether a low pH selection step alone or combined to UV mutagenesis could improve the viability of an acid sensitive *Bifidobacterium* strain, *B. breve* 99, in low pH food matrices. Furthermore, the potential of carriers and an oat fiber preparation to further improve the stability was studied. The best performing low pH tolerant variants in the present study were generated by UV-mutagenesis with 70–700 µJ/cm² followed by incubation in growth medium at pH 4.5. The most promising variants regarding the low pH tolerance showed, in repeated tests with cells grown without pH control, about one Log-value better survival in pH 3.8 fruit juice after one week storage at 4 °C compared to wild-type *B. breve* 99. Cells grown with pH control, PDX formulated and then frozen showed poorer viability in low pH fruit juice than cells grown with no pH control. For frozen concentrates pH 3.8 was too stressful and no or small differences between the variants and the wild-
type strain were seen. The differences detected at pH 3.8 with the cells grown without pH control were also seen with the frozen concentrates at pH 4.5. Some improvement in the stability could be achieved by using a combination of trehalose, vitamin C and PDX as a freezing carrier material, whereas a significant improvement in the stability was seen when oat fibre was added into the fruit juice together with the frozen cells. Due to the initial very poor fruit juice tolerance of *B. breve* 99 the obtained improvement in the stability was not enough for commercial applications. However, the same methods could be applied to initially better performing strains to further improve their stability in the fruit juice (Saarela, *et al.*, (2011b).

This study optimized the conditions of *Lactobacillus casei* NRRL B-442 cultivation in cashew apple juice, as well as, determined the proper inoculum amount and fermentation time. Moreover, it was investigated the survivability ability of *L. casei* in cashew apple juice during refrigerated storage (4°C) for 42 days. The optimum conditions for probiotic cashew apple juice production were initial pH 6.4, fermentation temperature of 30 °C, inoculation level of 7.48 Log
cfu/ml (L. casei) and 16 h of fermentation process. It was observed that the L. casei grew during the refrigerated storage. Viable cell counts were higher than 8 Log cfu/ml throughout the storage period (42 days). The values of lightness, yellowness and total color change increased and the values of redness reduced along the fermentation and refrigerated storage periods. The fermented juice with L. casei is a good and healthy alternative functional food containing probiotics. Cashew apple juice showed to be as efficient as dairy products for L. casei growth (Ana Lúcia, et al., 2011).

A study was conducted by Beatrice, et al. (2012) was aimed at determining the probiotic potential of a large number of autochthonous lactic acid bacteria isolated from fruit and vegetables. Survival under simulated gastric and intestinal conditions showed that 35% of the strains, mainly belonging to the species L. plantarum maintained high cell densities. Selected strains did not affect the immune-mediation by Caco-2 cells. All strains stimulated all 27 immune-mediators by peripheral blood mononuclear cells (PBMC). A significant ($P < 0.05$; $P < 0.01$) increase of the major part of cytokines and growth factors was found. A few
chemokines were stimulated. Immune-mediators with pro-inflammatory activity (IL-17, EOTAXIN and IFNγ) were significantly ($P < 0.01$) stimulated by all strains, followed by IL-1b > IP-10 > IL-6 > MIP1α. Stimulation of IL-12, IL-2 and IL-7 was strain dependent. Only a few strains increased the synthesis of cytokines with anti-inflammatory activity. Six $L. plantarum$ strains were further selected. Four were defined as the strongly adhesive strains (more than 40 bacteria adhering to one Caco-2 cell), and 2 as the adhesive strains (5–40 bacteria adhering to one Caco-2 cell). Five strains grew and acidified chemically defined medium with fructooligosaccharides (FOS) as the only carbon source. End-products of FOS fermentation were found. All strains inhibited enterohemorrhagic $E. coli$ K12 and $Bacillus megaterium$ F6 isolated from human sources. The results of this study showed that some autochthonous lactic acid bacteria from raw fruit and vegetables have functional features to be considered as novel probiotic candidates.

Espírito Santo do et al. (2012a) investigated the effect of the addition of passion fruit peel powder (PFPP) on the fermentation kinetics and texture parameters,
post-acidification and bacteria counts of probiotic yoghurts made with two milk types were evaluated during 28 days of storage at 4 °C. Milks were fermented by *St. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (CY340), and one strain of probiotic bacteria: *L. acidophilus* (L10 and NCFM), *Bif. animalis* subsp. *lactis* (Bl04 and HN019). The addition of PFPP reduced significantly fermentation time of skim milk co-fermented by the strains L10, NCFM and HN019. At the end of 28-day shelf-life, counts of *B. lactis* Bl04 were about 1 Log cfu/ml higher in whole yoghurt fermented with PFPP regarding its control but, in general, the addition of PFPP had less influence on counts than the milk type itself. The titratable acidity in yoghurts with PFPP was significantly higher than in their respective controls, and in skim yoghurts higher than in the whole ones. The PFPP increased firmness, consistency (except for the NCFM strain of *L. acidophilus*) and cohesiveness of all skim yoghurts. The results point out the suitability of using passion fruit by-product in the formulation of both skim and whole probiotic yoghurts.

A study conducted by Espírito Santo do et al., (2012b) evaluated the effect of the supplementation of
total dietary fiber from apple, banana or passion fruit processing by-products on the post-acidification, total titratable acidity, bacteria counts and fatty acid profiles in skim milk yoghurts co-fermented by four different probiotics strains: *L. acidophilus* L10 and *Bif. animalis* subsp. *lactis* BL04, HN019 and B94. Apple and banana fibers increased the probiotic viability during shelf-life. All the fibers were able to increase the short chain and polyunsaturated fatty acid contents of yoghurts compared to their respective controls. A synergistic effect between the type of fiber and the probiotic strain on the conjugated linoleic acid content was observed, and the amount of α-linolenic acid was increased by banana fiber. The results of this study demonstrate, for the first time, that fruit fibers can improve the fatty acid profile of probiotic yoghurts and point out the suitability of using fibers from fruit processing the by-products to develop new high value-added fermented dairy products.
It is opined by Martins et al. (2013) that the consumers are more aware and concerned about their lifestyle than ever before. This has increased demand for foods that promote health and wellness, such as functional products containing probiotic microorganisms, which have beneficial effects on the balance of intestinal microbiota. Among probiotic microorganisms, those of the Lactobacillus genus are the most commonly used by the food industry. Fermented dairy products are generally good food matrices for probiotics, but the consumption of these products is limited due to growing vegetarianism and the large number of individuals who are lactose intolerant or on cholesterol-restricted diets. Thus, the development of non-dairy probiotic products, including food matrices based on fruit, vegetables and cereals, has been widely studied. This paper reviews the main applications of probiotic microorganisms in products of vegetable origin and the characteristics that enable the use of these food matrices as potential carriers of probiotic bacteria.
Beverages can be a refreshing way for consumers to get an increasing range of health-promoting ingredients. The main objective of this investigation was utilizing whey in production of functional beverage as a whey-based fluid. Whey was fortified with mango powder (20.0%), flaxseed oil (0.5%) and (pectin JMJ and monoglyceride, 0.5% w/w), homogenized, pasteurized and then kept cool. Beverages were monitored at specific time intervals over a 15 day storage period. Physical properties and chemical composition was tested. Total Antioxidant Capacity (TAC), were analyzed using three different assays, i.e., DPPH free radical scavenging activity, Trolox Equivalent Antioxidant Capacity (TEAC) and Ferric Reducing-antioxidant Power (FRAP). These assays, based on different chemical mechanisms, were selected to take into account the wide variety and range of action of antioxidant compounds present in beverage. Results showed a new functional beverage with special characteristics has a little sedimentation and low viscosity exhibit excellent flavor derived from mango. This functional beverage increased its nutrient content including omega 3, minerals, vitamins and
unique proteins, besides digestible carbohydrates. The polyphenol-rich and carotenoids in beverage displayed high antioxidative capacities. Beverage was rich in polyphenol and carotenoids exhibited high antioxidative capacities. This whey-based beverage is put a wide range of components acting together synergistically that role, maintain and improve of a consumer health (Gad et al., 2013).

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