Review of Literature
II. REVIEW OF LITERATURE

One of the major challenges for dairy sector is to develop dairy foods that promote health and well-being. Today the perception towards food has changed and now food is perceived not only a source of nutrition but also as therapeutic agent. Studies in the clinical nutrition and pathology have successfully established interrelationship between food intakes, gut microbial ecology and human health. Human intestinal tract is home to trillions of bacteria comprising hundreds of beneficial species. The friendly organisms are called probiotics, which means "for life". Probiotics are "live microbial food supplements that have beneficial effect on the host by improving its intestinal microbial balance" (Fuller, 1989; 1997). The microbial ecology in the GIT influences many functions in our body such as digestion, absorption of nutrients, detoxification and ultimately the functioning of our immune system. All these aspects make the gut a target organ for the development of functional foods that can help in maintaining the relative balance of microorganisms in the GIT. Establishment of the microbial balance by shifting it towards a beneficial one with the help of specific dietary components like probiotics, prebiotics and synbiotics have opened the gateway for the development of functional foods. Once a functional food preparation is ready the next thing that is of prime importance is the shelf-life of these products. So, the technology for extending the shelf-life of health promoting foods needs to expand. To increase the keeping quality of perishable foods, various methods have been studied and are under study. One such technique is spray drying of high moisture containing products, especially the dairy products. Besides increasing the shelf-life of the food products, drying also make it a convenience food which can be by made ready to use and can be carried easily.
The present review is focused mainly on the history, health benefits associated with plain and probiotic yoghurt, further increasing the health promoting effects of yoghurt with probiotic and whey protein concentrates (WPC). Further it covers the probiotic, hurdles in production of probiotic yoghurt, ingredients, processing of concentrated yoghurt and effect of microwave treatment. It also covers the use of spray drying, different packaging material with modified atmospheric packaging to increase the shelf-life of protein enriched probiotic yoghurt powder.

2.1 History

Acidification of milk by fermentation is one of the oldest method of preserving milk, for imparting special favourable organoleptic qualities. There are different methods of carrying out this fermentation in various parts of the world which has given rise to a range of fermented milk products including Kumiss, Kefir, Acidophilus milk and Yoghurt. These products vary considerably in composition, flavour and texture according to the nature of fermenting organisms, the type of milk used and the manufacturing process (Kosikowski, 1977).

The origin could be traced to the Middle East and the term Yoghurt is derived from the Turkish word called "Jugurt". It is called by various names in different parts of the world viz., Labneh (Middle east), Zabady (Egypt), Matzooa (USA) and Dahi in India (Tamime and Robinson, 1985). The process of yoghurt making is an ancient craft which dates back to thousands of years, but it is assumed that prior to nineteenth century the various stages involved in the production of yoghurt were little understood. The uniqueness of yoghurt is attributed to the symbiotic fermentation (Vedamuthu 1991).
2.1.1 Varieties and classifications

Variability between types of yoghurt stems from the ingredients, how they were made, and what has been added (Tamime and Robinson 1999). Various processing steps can affect flavour and texture. Yoghurt can be made from non-fat, low-fat, and full fat milk, or additional cream can be added to yield even higher milk fat contents. Protein and carbohydrate stabilizers can affect both flavour and texture (Chandan, 2006). Vitamins and minerals can be added to fortify yoghurts, and preservatives may be added to further lengthen their shelf-lives.

2.2 Definition

According to IS:7035 (1973) yoghurt is a cultured product obtained by using *S. salivarius* subsp. *thermophilus* and or *L.delbrueckii* subsp. *bulgaricus*. The product should not exceed 0.8 per cent lactic acid while yeast and mould counts not exceeding 100/g, and coliform count of not more than 10/g. The product should be negative for phosphatase test.

According to FAO/WHO (1976) "Yoghurt is a coagulated milk product obtained by lactic acid fermentation of milk through the action of *S.salivarius* subsp. *thermophilus* and *L.delbruecki* subsp. *Bulgaricus* with or without addition of whole milk powder or skim milk powder or whey powder. The desirable microorganisms in the final product must be viable and abundant”.

In the United States, the definition and regulation governing yoghurt are set by the Food and Drug Administration (FDA, 1991). According to that "Yoghurt is produced by
culturing cream, milk, partially skimmed milk or milk either alone or in combination with lactic acid producing bacteria viz., *L. bulgaricus* and *S. thermophilus*. The regulations specify that yoghurt before addition of bulky flavours contains not less than 3.25 per cent milk fat, 8.25 per cent milk solids-not-fat and a titrable acidity of 0.9 per cent expressed as lactic acid.

The Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) set broader international standards for yoghurt in the *Codex Standard for Fermented Milks* (2003). This document simply requires that yoghurt be the result of a fermentation by *Streptococcus thermophilus* and *Lactobacillus delbrueki* ssp. *bulgaricus* cultures, and contain a minimum of 2.7% milk protein, less than 15% milk fat, and at least 0.6% titratable acidity. If a claim regarding live microorganisms is made on the package, the Codex specifies that at least $10^6$ colony forming units (CFU) per gram must be present.

### 2.3 Nutritional and Therapeutic Properties of Plain and Probiotic Yoghurt

In recent years there is a considerable increase in the consumption of cultured dairy products especially yoghurt which could be ascribed to its wholesomeness in terms of nutritional and therapeutic properties (Shahani and Chandan, 1979). The starter cultures used in cultured dairy products bring about changes in physico-chemical characteristics besides enhancing the nutritional value of the resultant product. Hydrolysis of lactose to lactic acid together with acetaldehyde impart characteristic odour and taste to yoghurt. The fermented product is highly recommended to the lactose
intolerants because of the reduced lactose content. Besides this, lactic acid also helps in the absorption of calcium and phosphorous in the intestine (Renner, 1986; Kaup, 1988).

Yoghurt is an excellent source of protein. Consumption of 200 to 250 ml of yoghurt meets the minimum daily protein requirement of an adult. This protein is highly digestible as most of the protein is in the digested form (Deeth and Tamime, 1981). Consumption of yoghurt promotes growth as a result of improved lactose digestion, greater mineral absorption besides providing thiamine, riboflavin, niacin and folic acid (Renner, 1986). The antimicrobial properties of yoghurt are well established (Shahani et al., 1974; Shahani and Chandan, 1979; Renner, 1983). The antimicrobial properties have been found to be effective against pathogenic organisms especially Gram negative intestinal bacteria. Epidemiological and dietological studies have also shown that consumption of dairy products fermented by lactobacilli reduce the risk of colon cancer in both animals and humans (Renner, 1986). Even some of the specific strains of lactobacilli have been demonstrated to be effective in limiting a number of transplanted and chemically induced cancers (Tomar and Prasad, 1989). The more recent studies have revealed that yoghurt cultures are capable of controlling intestinal disorders and blood cholesterol levels (Rao, et al., 1994).

The addition of probiotic cultures to milk and milk products inhibits the growth of different pathogenic microorganisms by producing some bacteriostatic molecules and many bioactive substances, e.g. bioactive peptides, free fatty acids, free amino acids and oligosaccharides (prebiotics) during fermentation and storage. The addition of probiotic cultures (*Bifidobacterium longum, B. bifidum, B. infantis*, *L. acidophilus* and *L.*
delbrueckii ssp. bulgaricus) inhibits the growth of pathogenic bacteria (Sartor, 2005). A number of studies have shown that yoghurt has inhibitory effect on enteropathogens. Cholesterol lowering and immunomodulatory properties of fermented milk have been reviewed by Hosono et al., (2002). Milk and cultured milk have a definite hypocholesterolemic effect on the consumer and leads to steady decrease in the blood triglyceride level (Chawla, 1982).

Vijayendra (1994) developed direct bulk freeze-dried starter culture formulation for probiotic dahi and yoghurt. The in-vivo study was conducted to evaluate the probiotic effect of these products, containing Bifidobacterium spp. and L. acidophilus strains and was found to show probiotic properties. Sodini et al., (2004) studied the effect of storage on probiotic cell counts in fermented milk processing. They reported that storage of fermented milk containing probiotic cultures (L. acidophilus and L. rhamnousu) significantly increased the probiotic cell count. Kailasapathy and Rybka (1997) successfully carried out the incorporation of L. acidophilus and B. bifidum in yoghurt to produce Acidophilus-Bifidum (AB) yoghurt having therapeutic properties and studied the probiotic attributes of AB yoghurt. AB yoghurt has been reported to attenuate diet induced hypercholesterolaemia and deposition of cholesterol and triglyceride in liver and improves antioxidant status (Rajpal, 2006). Probiotic yoghurt prepared with L. acidophilus and L. casei was found to have strong tumor inhibitory and hepato-protective activities (Singh, 2007). Probiotic yoghurt may delay the progression of chemical and dietary-induced diabetes (Yadav et al., 2006). It has also been reported that yoghurt prepared with Lactobacillus delbrueckiissp. bulgaricus, Streptococcus salvariussssp. thermophilus and Lactococcus lactissssp. lactisbiovar. diacetylactis, inhibits angiotensin-I
converting enzyme in hypertensive rats and reduces systolic blood pressure in hypertensive humans in the age group of 45 to 55 years (Ashar and Chand, 2004a;b;c). Probiotic lactobacilli appeared to increase the production of FFAs by lipolysis of milk fat, and produced CLA by using internal linoleic acid, which may confer nutritional and therapeutic value to the product (Yadav et al., 2007).

2.4 Probiotic and Their Health Benefits

The concept of probiotics was evolved at the turn of the 20th Century from a hypothesis proposed by Russian Scientist, Elie Metchnikoff, that the long healthy life of Bulgarian peasants was resulted from the consumption of fermented milk products. According to Metchnikoff (1908), the regular consumption of live beneficial bacteria such as lactic acid bacteria (LAB) through fermented dairy products are needed to maintain a good equilibrium of the intestinal micro flora that minimize putrefactive microbial fermentations. The word "Probiotic" is derived from the Greek, which means "for life" and first used by Lilly and Stillwell (1965) to describe substances secreted by microorganism, which stimulate the growth of another. Parker (1974) defined probiotic as organism and substances, which contribute to intestinal microflora. Fuller (1989) improved Parker's definition of probiotic with the following distinction: “A live microbial feed supplement which beneficially affects the host animal by improving its intestinal by improving its microbial balance.” This revised definition emphasizes the requirement of viability for probiotics and introduces the aspect of a beneficial effect on the host, which was, according to his definition, an animal. Havenaar et al., (1992) widened the definition of probiotics with respect to host and habitat of the microflora as follows: "A viable mono or mixed culture of microorganisms which applied to animal or man beneficially
affects the host by improving the properties of the indigenous microflora". According to Salminen et al., (1998), a probiotic is "a live microbial culture or cultured dairy product which beneficially influences the health and nutrition of the host". According to Schaafsma (1996), "Oral probiotics are living microorganisms which upon ingestion in certain numbers exert health effects beyond inherent basic nutrition". According to WHO/FAO (2002), live microorganisms which when administered in adequate amounts confer a health benefit on the host. According to Schrezenmeir and De Vrese (2001), probiotic bacteria are live microbial strains that, when applied in adequate doses, beneficially affect the host by improving its intestinal microbial balance.

Probiotic foods are products that contain a living ingredient in sufficient concentration, so that after their ingestion, the assumed effect is obtained. Probiotics have been used to treat diarrhea, tooth decay (*Lactobacillus* GG), vaginitis, yeast infection, canker sores, Grohn's disease (*Saccharomyces boulardii*), eczema; food allergies, HIV support (*Saccharomyces boulardii*), immune function, infection, acute pancreatitis (*Lactobacillus plantarum*), ulcerative colitis, and chronic candidiasis etc. (Pedigone et al., 1999). In a meta-analysis of eighteen studies, workers have reported that probiotics can reduce the duration of acute diarrhea, especially of rotavirus in children (Huang et al., 2002). If the probiotics are given to mother before childbirth and to child after birth it reduces the risk of allergic diseases in children with family history of allergy (Marschan et al., 2008). In-vitro study showed that probiotic strains used in commercial products might affect the oral ecology by specifically preventing the adherence of other bacteria and by modifying the protein composition of the salivary pellicle (Haukioja et al., 2008). It has been reported that high-dose probiotic and prebiotic co-therapy can be safely and
effectively used for the treatment of active Crohn's disease (Fujimori e.t al., 2007). Studies were conducted with conventional and probiotic yoghurt and concluded that both enhanced the stimulated production of pro-inflammatory cytokines (Meyer et al., 2007).

Prescott et al., (2008) suggested that supplementation with probiotics in pregnancy has the potential to influence fetal immune parameters as well as immunomodulatory factors in breast milk. It has been reported that a multi-strain probiotic supplement may benefit patients with irritable bowel syndrome (Williams et al., 2008). It has been proposed that probiotics can be used in association with standard anti -H. pylori treatment, as they are able to improve patient compliance by reducing antibiotic-related adverse events, thus increasing the number of patients completing the eradication therapy (Franceschiet al., 2007).

A number of beneficial roles of probiotic strains have been reported (Havenaar and Huisin't Veld, 1992; Lee and Salminen, 1995; Salminen, 1990). These includes

- Re-establishment of balanced intestinal micro flora
- Improving Colonization resistance and/or prevention of diarrhea
- Systematic reduction of serum cholesterol
- Reduction of fecal enzymes and potential mutagens
- Metabolism of lactose and reduction of lactose intolerance
- Enhancement of immune system response
- Improved $Ca^{2+}$ absorption
- Synthesis of vitamins and pre-digestion of proteins
Probiotics have been suggested to have the following properties and functions: (i) adherence to host epithelial tissue, (ii) acid resistance and bile tolerance, (iii) elimination of pathogens or reduction in pathogenic adherence, (iv) production of acids, hydrogen peroxide and bacteriocins antagonistic to pathogen growth, (v) safety, non-pathogenic and non-carcinogenic, and (vi) improvement of intestinal microflora, (vii) have generally regarded as safe (GRAS) status, (viii) normal inhabitants of intestinal tract of human, (ix) technically suitable for process application. (Fuller, 1989; Kaur et al., 2002; Ouwehand et al., 2002).

Common probiotics include 1) lactobacilli such as L. acidophilus, L. johnsonii, L. casei, L. delbrueckiissp. bulgaricus, L. reuteri, L. brevis, L. cellobiosus, L. curvatus, L. fermentum, L. plantarum; 2) gram positive cocci such as Lactococcus lactis ssp. cremoris, Streptococcus salivarius ssp. thermophilus, Enterococcus faecium, S. diacetylactis, S. intermedius; and 3) bifidobacteria such as B. bifidum, B. adolescentis, B. animalis, B. infantis, B. longum (Mercenier et al., 2002). Also other microbial species, besides lactic acid bacteria (LAB) like Bacillus subtilis, Propionibacterium spp. and yeasts (Saccharomyces boulardii) have been accepted and used as probiotics (Jan et al., 2001).

The human intestinal tract constitutes a complex ecosystem. The intestine of an infant is sterile but soon after birth, a variety of bacteria starts colonizing the infant intestinal tract. The first microorganisms to appear in the colon of newborns are usually Enterobacteriaceae and enteric streptococci. Microorganisms such as Lactobacillus, Clostridium, Bacteriodes, and Bifidobacterium appear within the first week of life. In breast-fed infants, it is common for counts of bifidobacteria to reach 10^{10} - 10^{11} cfu/gm of
feces (Modler et al., 1990). The increase in bifidobacteria results in lactococci, enterococci and coliforms representing less than 1% of the intestinal population, while bacteroides and clostridia normally become absent. Formula-fed infants normally have higher levels of enterobacteriaceae, streptococci, and other putrefactive bacteria and one log-less of bifidobacterial counts. This suggests that bifidobacteria could be offering resistance to infections in breast-fed infants due to their higher counts (Lourens-Hattingh and Viljoen, 2001). With weaning and ageing, slow but sure changes in the intestinal flora contour occur resulting in an adult type micro flora in which bifidobacteria becomes the third common genus in the intestinal tract. At an older age, bifidobacteria decrease while populations of clostridia, including C. perfringens, lactobacilli, streptococci and enterobacteriaceae increase significantly (Mitsuoka, 1992).

Historically, humans were exposed to probiotics by the means of fermented foods. However, today's modern diet contains very less numbers of fermented foods. Moreover, the increased hygiene measures in food manufacturing plants and restaurants have resulted in humans being exposed to as few as one millionth of the probiotic organisms to which their ancestors were exposed (Markowitz and Bengmark, 2002). Ageing, increased stress and a hectic lifestyle have further contributed to the declining populations of probiotic organisms in the human gut (Lourens-Hattingh and Viljoen, 2001). With today's changing life style and food habits, it becomes vital to complement human diet with adequate doses, of probiotic microorganisms to re-establish the intestinal micro flora balance and help maintain good health. The balance of intestinal flora can be altered in favor of potentially harmful bacteria by a number of factors such as peristaltic disorders, cancer, surgery, liver, kidney or immune disorders, radiation therapy, stress, diet,
antibiotics and ageing. When harmful bacteria dominate the intestinal flora, essential nutrients may not be produced and the level of damaging substances, including carcinogens, putrefactive products and toxins may increase (Mitsuoka, 1996). Thus manipulating the composition of intestinal micro flora by introducing live bacteria or stimulating growth of certain bacterial population groups can prevent harmful effects and promote beneficial actions of the intestinal micro flora. It is on this basis that probiotics were first introduced. Administration of probiotic bacteria can hence be useful to treat intestinal disorders and to prevent those (Salminen et al., 1996). As a result, recently, probiotics have been marketed as dietary supplements in the form of tablets, capsules and freeze-dried preparations medium. An empty stomach has a low pH that destroys most bacteria. When food is ingested, the pH in the stomach quickly rises and probiotic bacteria can easily pass unharmed to the small intestine where they are most effective (German et al., 1999).

Probiotic foods offer a low cost dietary component that has the potential to promote health of the consumer in a variety of ways (Goldin, 1998). Probiotics, upon ingestion, exert many health benefits beyond inherent nutrition, and should be at the level of $10^6$-$10^7$ live microorganisms per gram of product at the time of consumption (Guarner and Schaafsma, 1998; Ouwehand and Salminen, 1998; Shah, 2000). These high numbers have been suggested to compensate for the possible loss in the numbers of probiotic organisms during passage through the stomach and intestine. To exhibit their health benefits; the probiotics must reach to the intestine in sufficient numbers. Therefore, the minimum count of probiotic bacteria should be $10^6$ CFU/g of the product at the expiry date (Kurmann and Rasi, 1991). The National Yoghurt Association (NY A) of the United
States specifies that in order to use the NY A "Live and Active Culture" logo on the container of the products, there should be $10^8$ CFU/g lactic acid bacteria at the time of manufacture (Lourens-Hattingh and Viljoen, 2001). Similarly, the Australian Food Standards Code regulations require $10^6$ CFU/g of viable lactic acid cultures used for yoghurt fermentation (ANZFA, 2003). In Japan, the Fermented Milk and Lactic Acid Beverages Association has specified that there should be at least $10^7$ CFU/ml of viable bifidobacteria in fermented milk drinks (Lourens-Hattingh and Viljoen, 2001). The International Dairy Federation (IDF) requires $10^7$ CFU/ml of L. acidophilus in products such as acidophilus milk and $10^6$ CFU/g of bifidobacteria in fermented milks containing bifidobacteria at the time of sale (IDF, 1992). Researchers have demonstrated that enteral feedings with added probiotics can reduce the severity of acute pancreatitis (Muftuoglu et al., 2006).

The major group of bacteria which constitute probiotic is the lactic acid bacteria (LAB). The antimicrobial effect of LAB is mainly due to their lactic and organic acid production, causing the pH of the growth environment to decrease (Kuipers et al., 2000). Low pH induces organic acids to become lipid soluble and diffuse through the cell membrane into the cytoplasm (Gottschalk et al., 1989). LAB also produce acetaldehyde, hydrogen peroxide, diacetyl, carbon dioxide, polysaccharides and bacteriocins (Rodriguez et al., 2003), some of which may act as antimicrobials. LAB synthesizes enzymes, vitamins and antioxidants, some of which may act as antimicrobials. LAB synthesizes enzymes, vitamins, antioxidants and bacteriocins. With these properties, intestinal LAB constitutes an important mechanism for the metabolism and detoxification of foreign substances entering the body (Salminen, 1990). Both Lactobacillus spp. and
Bifidobacterium spp. have been accorded the GRAS status because of their long history of safe use in foods (Salminen et al., 1998). Clinical studies have also shown health improvements associated with consumption of probiotics including reduction in the incidence of childhood atopic eczema (Kalliomaki et al., 2001), decrease in rotavirus shedding in infants (Phuapradit et al., 1999) and reductions in antibiotic-associated diarrhea (Plummer et al., 2004). Gani Lone (2002) studied various strains of Lactobacillus spp. for their hypocholesterolemic effect and proposed that L. casei ssp. casei NCDC 19, L. acidophilus NCDC 14 and L. plantarum NCDC 20, have the maximum probiotic potential. Among the various milk products, the ideal vector for the delivery of probiotic bacteria to consumers is fermented milks and yoghurt. A growing number of yoghurt manufacturers are therefore incorporating Lactobacillus and Bifidobacterium spp. in their products (Lourens-Hattingh and Viljoen, 2001).

Evidence has been presented that some lactobacilli can directly stimulate the immune system on the gut mucosal surface via localized GI tract lymphoid cell foci (Perdigon et al., 1999). Morishita et al., (1971) demonstrated that intestinal origin LAB established in the digestive tract of germ-free chickens better than did non-intestinal LAB strains. A number of mechanisms work to prevent harmful bacteria from growing on and attaching to the intestinal epithelium: production and secretion of antimicrobial agents such as bacteriocins and organic acids (Reid, 2001), adherence via competition for the binding sites and steric hindrance (Bezkorovainy, 2001) and barriers interfering with pathogens and hence promoting the elimination of harmful bacteria. In addition, probiotics have been shown to be able to prevent the development of diabetes which is highly linked to obesity (Sofia et al., 2010).
2.4.1 *Bifidobacteria*

Bifidobacteria were first isolated in 1899 from the faeces of breastfed infants by Tissier of the Pasteur institute. When first isolated the organism was named bacillus bifidus, based on the morphology as the organism typically exists in a Y shaped or ‘bifid’ form. Bifidobacteria are claimed to provide several prophylactic probiotic and therapeutic benefits. Intestinal contents have viable microbial count of about $10^{12}$ cfu g$^{-1}$. Bifidobacteria are gram-positive nonmotile, non-sporeforming and anaerobic organisms (Desponde, 2007) The importance of bifidobacteria should not be underestimated especially in small children and breast feeding is found to promote bifidobacterial growth in the intestine compared to standard formula feeding (Harmsen, *et al.*, 2000). The bifidobacterial growth in protein adjusted UF milk with lactose and honey showed improved colony count. The counts were 8.95 log cfu after 20th hour. (Suresh *et al.*, 2005). Bifidobacteria are predominant in the newborn and have been known as one of the major groups of saccharolytic bacteria in the human colon, constituting up to 25% of the total population in the adult colon. (Wang *et al.*, 1999). Supplementation of bifidobacteria has been shown to influence immunoparameters such as stimulation of local IGa-production (Amster *et al.*, 1994) as well as other beneficial effects such as synthesis of folate (Mattila-Sandholmet *et al.*, 2002) are well known.

2.4.2 *Lactobacillus acidophilus*

Lactobacilli are one of the most commonly recognized species of the genus *Lactobacillus*. This was first proposed in 1901 by MW Beijerinck. The species *acidophilus* (meaning acid loving) was perhaps so named because, historically,
lactobacilli isolated from the intestinal tract of humans and animals were often called acidophilus (implying ‘acidic gastric’ environment) based on morphological and physiological characteristics. Morphologically, *Lactobacillus acidophilus* group are gram-positive, non-sporeforming rods with rounded ends generally 0.6-0.9 x 1.5-6µm, which occurs singly in pairs and short chains. These are also found to stimulate an immune system against the unwanted microflora, improve milk digestibility and help in controlling serum cholesterol levels. The bacterium also possesses anticarcinogenic properties. (Mathur, 1993).

Studies suggest that *Lactobacillus jonshnii* LA-1 balances the gastrointestinal microflora, enhances immune system and acts as adjuvant in Helicobacter pylori treatment. It is the normal inhabitant of lower end of the small intestine and in this niche the species both occupies the lumen of gut and adheres to the surface of the intestinal wall. Growth of *Lactobacillus acidophilus* may occur at temperature as high as 45 ºC but optimum growth occurs within 35-40ºC its acid tolerance varies from 0.3 to 1.9 per cent titratable acidity (Gomes and Malcatta, 1999). And most of them attained maximum growth at 24 h of incubation at 37ºC. Maximum viable count of 9.2 logcfu/ml was recorded for *Lactobacillus acidophilus* (Borpuzari et al., 2007).

### 2.5 Hurdles Affecting Development of Probiotic Fermented Milks and Yoghurt

Milk and the derived products like fermented milk are the primary carriers of lactobacilli for delivery to host. Incorporation of probiotic cultures into milk and fermented milk products is easy. The probiotic bacteria can be maintained as live or freeze dried in products such as sweet acidophilus milk, which can stay for many months,
however, consumers are preferring to take probiotics in the form of fermented foods and beverages such as cultured milk, because of their taste, texture and association with good health (Lee and Salminen, 1995). Cultured milk is more digestible than the milk (Gilliland et al., 1985). Consuming cultured milk products containing lactobacilli species allow continuous passage of these organism through gut (Tamura, 1983). For manufacture of such products, not only the probiotic effect of selected strain is considered but also the ability of the probiotic culture to multiply in milk, their survival during product manufacture and storage under refrigerated conditions in acidic milk and impact on the sensory attributes of the finished product are also taken in to consideration (Klaver et al., 1993). During technological processing bacteria cells are exposed to different stresses (Mattila et al., 2002).

The European market, Australia and Japan is flooded with the probiotic products. There are several probiotic products in developed country because of their impact on health and nutritional requirement. The name of few popular probiotic products are Yakult, Leben, Probiotic cheese, Yoghurt, Bio-kefir, Probiotic Ice cream (Dos Pinos). The predominant organism in the probiotic products is *L. acidophilus*, *L. casei*, *L. paracasei*, *L. plantarum*, *Streptococcus thermophilus*, *L. bulgaricus*, *Bifidobacterium* species etc. The Japanese industry has pioneered R&D in these sectors and has one of the largest growing markets for probiotic and functional foods. The Australian probiotic segment accounts for 15% of all yoghurt produced in the country and its growth rate has been at 25% while total growth of Yoghurt is at 3.5 %. Yakult Hansa Co. Ltd., which manufactures and markets Yakult, has seen growth in its area and the product is now available in more than twenty European countries.
Probiotic products are helpful for lactose intolerance people and prevent from cancer, reduce the cholesterol level and perform several other health benefits for the consumer. In India, Lassi is one of the preferred fermented milk products; it could be exploited as a carrier for delivering probiotic microorganisms. In a study, it was found that the consumption of probiotic containing yoghurt improved the mood of those whose mood was initially poor (Benton *et al.*, 2007).

### 2.6 Whey Protein Concentrate (WPC)

Whey is now a way for health in light of the benefits that are imparted by whey proteins (Alok and Kanawajia, 2010). Whey is the largest by-product of the dairy industry. It is obtained during the manufacture of cheese, casein, paneer, chhana and shrikand. Which are very popular and great market demands especially for cheese as its consumption is steadily increasing due to changing food habits. Normal bovine milk contains about 3.5 per cent of protein of which casein constitutes 80% and whey proteins 20 per cent per cent. Liquid whey, contain approximately 20 per cent of the original proteins of milk ranging from 4-7g/l (Marshal, 1982). Biological value of whey proteins superior to most other proteins, whey proteins also have a high content of sulphur-containing amino acids, which support antioxidant functions (RichaSinha *et al.*, 2007). Presently, whey protein concentrate (WPC) constitutes very small proportions (10%) of protein utilization in food industry. More product formulation work is needed to move WPC in to the general market place (Narendare *et al.*, 2005).
2.6.1 Nutritional Significance of Whey Proteins

In 1993, the US Food and Drug Administration adopted the Protein Digestibility Corrected Amino Acid Score (PDCAAS). According to the analysis of FAO/WHO experts, whey proteins possess higher nutritional value because of higher concentrations of essential amino acids such as lysine, tryptophan, isoleucine, threonine, etc. (Renner, 1983), and has high nutritional value as they contain a relatively high proportion of branched chain and essential amino acids (Ha and Zemel, 2003). Pasim and Miller (2000) reported that Biological Value (BV), Net Protein Utilization (NPU), Protein Efficiency Ratio (PER) and Protein Digestibility Corrected Amino Acids’s are also higher (104, 92, 3.6, 1.6) as against casein (77, 76, 2.9, 1.0), whole egg (100, 94, 3.8, 1.0) and cow milk (91, 82, 3.1, 1.0), respectively.

Whey contains many nutritionally rich constituents among all the constituents. Whey proteins comprised of 61.4 per cent \( \beta \)-lactoglobulin, 20.5 per cent \( \alpha \)-lactalbumin, 6.0 per cent serum albumin and 12.2 per cent immunoglobulin. Whey proteins are one of the highest quality natural proteins available (Patel et al., 1991). Owing to their excellent nutritional and functional properties, whey concentrate has been seriously considered as a nutritional ingredient to improve nutritional richness of variety of foods (Huffman, 1996). Compared to the various sources of protein such as soya, beef, egg, casein etc., whey proteins are excellent both in terms of nutritional and economic point of view. They also exhibit multidimensional functionality (Mann, 1998; Jayaprakasha and Brueckener, 1999) proportionately more sulphur containing amino acids (cysteine and methionine) than caseins, which contributes to the higher PER of whey proteins (3.2) than casein
Any protein with a PER of 2.5 or more considered as good quality (Walzem, et al., 2002).

### 2.6.2 Functional Properties of Whey Proteins

Today food industries are looking for ingredients, which can provide good functional and nutritional properties for formulations of value added food products. Whey proteins provide good functional and nutritional properties for formulation novel products which have potential to improve the quality of food products. Most of the key functional properties may be classified into two main groups: hydration related and surface related (Morr and Ha, 1993). Hydration related functional properties include dispersability, solubility, swelling, viscosity and gelation. Surface related properties include emulsification, foaming, and absorption at air water interfaces (Dewit, 1989; Kinsella and Whitehaed, 1989; Jayaprakasha and Brueckner, 1999). The presence of WPC with high protein content (WPC 80) resulted in a decrease in the firmness and consistency and an increase in the cohesiveness of yoghurt. The functional peptides derived from milk serve to modulate processes such as digestion, circulation, immunological responsiveness, cell growth and repair of nutrient uptake. Opioid agonistic, opioid antagonistic, antihypertensive, immunomodulatory, antimicrobial, antithrombic and mineral binding activities (Nayak et al., 1999).

### 2.6.3 Whey Protein Concentrates (WPC) in Plain and Probiotic Yoghurt

The present consumer demand is for low calorie, high protein yoghurt i.e., low fat or nil fat yoghurt. Normally such yoghurt acceptability is impaired by poor consistency and syneresis (Costa-Antunes et al., 2004). A low calorie high protein yoghurt very much
comparable to full cream yoghurt can be prepared by replacing 25% of SMP with WPC; which possessed very much comparable sensory and textural properties viz., hardness, springiness, cohesivity, gumminess and syneresis with better acetaldehyde flavor retention during storage (Morris et al., 1995; Diana et al., 2003; Augustin et al., 2003). Kailasapathy et al., (1996) reported that 20% replacement of SMP with WPC in low fat yoghurt had improved buffering capacity, microstructure and suffered less syneresis due to better protein network.

Guzman-Gonzalez et al. (1999) studied the effect of use of different dried dairy products viz., WPC, SMP, milk protein concentrates (MPC) and skim milk concentrate (SMC) on set type of low fat yoghurt and reported that low fat set yoghurt prepared with WPC had lower viscosity, softer body and suffered less syneresis than two control yoghurts prepared using SMP or SMC alone and suggested that low fat yoghurt made with WPC may be more suitable for preparation of low fat yoghurt drink (William et al., 2008).

The global market for probiotic products is estimated to be US$ 31.2 billion in 2014. In this segment probiotic dairy products have been enjoying the highest market share and are expected to continue to do so in future. Among the probiotic dairy products yoghurt is in the front line (Annon, 2009). Lot of research work had been carried out on the use of WPC in probiotic fermented dairy products in particular fermented milk and yoghurt. As reported by many scientists, use of WPC in probiotic yoghurt has got amply number of advantages as follows;
Improved sensory properties, superior flavor and improved shelf-life (Bozanic et al. 2000, 2001; Hekmat et al., 2004; Reid, 2001).

Low syneresis (Guzman-Gunzalezet al., 1999)

Improved growth and viability of probiotic organisms viz., *L. acidophilus* and *B. bifidum* (Tawfik et al., 2003; Martin-Diana et al., 2003; Bozanic et al. 2004; Janeret et al., 2004; Antunes et al., 2005).

Improved starter culture growth, rate of acidification, survivability and their enzymic activity in-vivo, which could help people with lactose mal-digestion to more readily consume dairy products (Kailasapathy et al., 1996; Bury et al., 2003; Christopher et al., 2006).

2.7 Spray Dried Fermented Milks, Yoghurt and Technological Problems of Drying

The spray drying of microorganisms dates back to 1941 when first attempt to dry cultures of lactic acid organisms were tried and $6.6 \times 10^8$ - $8.6 \times 10^9$ organisms per gram of powder were obtained. Starter cultures were dried in a laboratory size spray drier and their subsequent growth rates was measured as activity value (% acidity x 100) by Sapp and Hedrick (1960). It was observed that outlet-air temperature of 195°F or above strongly inhibited activity of the cultures. At a temperature of 170- 190°F, activity values were 45 to 70, optimum temperature was 136- 165°F, when dry cultures with activity values usually above 70 were obtained. Ivanovet al., (1973) and Suk-Shin-Kim and Bhowmik (1994) outlined the method for production of dried yoghurt and reported that pre-concentration of stirred yoghurt under vacuum didn’t had any effect on survivability of starter organisms. Poltevaet al., (1960), prepared spray dried sour milk of an antibiotic
resistant strain of *L. acidophilus* and the product was found to be valuable supplement for children suffering from infection of digestive tract and other illness. Many workers have studied spray drying of yoghurt and factors affecting the survival of starter organisms viz; inlet – outlet temperatures, phase of growth of organisms, improving survivability by different pretreatment methods, protective agents and reported that inlet temperature didn’t had much effect on survivability, outlet temperature of 65 – 75 °C is optimum and protective agents like hydrocolloids viz; stabilizing gums (sodium alginate, gum accecia etc.,) have improved the survivability by one log (Peri and Pompei, 1976; Suk-Shin-Kim and Bhowmik, 1994; Marthaet al.,2010; Peighambardoustet al., 2011). Banuet al.,2010 studied the effect of inlet-outlet and feed temperature on survivability starter organisms and reported that inlet 171°C, outlet 60.5°C and feed temperature of 15 °C are the optimum conditions for survival of starter organisms in spray drying of yoghurt. Selvamuthukumaran and Shukla (2006) have studied the production of spray dried bifidus milk powder and reported that inlet-temperature and air pressure has a significant effect on survival of bifidobacteria in the final product. Studies by Daemen and Van der Stege (1982), suggested that there is insignificant effect of inlet-air temperature on the destruction of bacterial cells and enzymes during spray drying. Bielecka and Majkowska (2000) performed studies on the effect of outlet-air temperature in the range of 60-80°C upon the survival of yoghurt cultures, as well as the moisture content and sensory properties of yoghurt powder. They reported that the survival of yoghurt cultures was highest at 60 and 65°C, but excessive moisture (10.2%) of yoghurt powder had a negative effect on its texture. Moisture content of the powder was low at 80°C (4.4%), but this temperature caused sensory faults in the reconstituted product and survival of bacterial
cultures also decreased considerably. Temperatures within the range of 70-75°C ensured satisfactory survival of yoghurt cultures (*L. delbrueckii* ssp, *bulgaricus* -13.7-15.8%; *S. thermophilus*: 51.6-54.7%), maintained proportion of the strains (L:S = 1:3), satisfactory moisture content (5.1-6.3%), and good sensory properties of yoghurt powder (Kumar and Mishra, 2004a; Walton, 2000). Kumar (1981) studied the preparation of spray dried acidophilus milk powder and reported that there is about 5-fold decrease in the count of viable cells after spray drying the acidophilus milk. When stored at -15°C and 5°C, stability of the viable cells was maintained in the powder but at higher temperatures, there was rapid destruction in the count of viable cells.

### 2.8 Effect of Spray Drying on Survival of Probiotic

For successful delivery in foods, probiotics must survive food processing and storage during product maturation and shelf-life (Stanton *et al.*, 2003). For the development of dairy-based functional foods containing high numbers of viable probiotics, the culture should have the ability to grow in milk-based media, remain viable and retain probiotic properties during production and storage (shelf-life) of the probiotic food product (Knorr, 1998; Saxelin *et al.*, 1999). However, the bacteria do not survive in high numbers during processing of dairy products (Dave and Shah, 1996; Hamilton-Miller *et al.*, 1999; Kailasapathy and Rybka, 1997).

Though freeze-dried powders and frozen concentrates of probiotic *Lactobacilli* and *Bifidobacterium* spp. have been developed, usage of spray drying as a means of probiotic culture preparation requires more attention. Few studies have demonstrated the potential of this approach for some strains including *L. acidophilus* cultures (Prajapati...
al., 1987), *L. paracasei* (Gardiner *et al.*, 1998, 2000; Desmond *et al.*, 2001; 2002; Kearney *et al.*, 1990), and other probiotics including *Bifidobacterium* spp. (O'Riordan *et al.*, 2001; Lianet *et al.*, 2002; Simpson *et al.*, 2005; Natalia *et al.*, 2010). Spray-dried powders harboring high levels of viable microorganisms provide a convenient form of these cultures for storage purposes and applications in functional food developments (Silva *et al.*, 2002). Although Spray drying is an economical process for, the large-scale preparation of cultures, it suffers from the disadvantage of causing bacterial cell injury and death, which are primarily due to the effects of heat and dehydration leading to destruction of the properties and performance characteristics of probiotic cultures (Teixeira *et al.*, 1995; To and Etzel, 1997). The major limitation of spray drying of probiotic cultures is the loss of viability that occurs during processing and storage of the powders (Daemen and Van der Stege, 1982; Gardiner *et al.*, 2000; Desmond *et al.*, 2001). Many lactic acid bacteria with probiotic properties have been reported and many methods for cell propagation and probiotic preparations have been described (Fuller 1989). In most cases, the cells are freeze-dried or spray-dried (Mattila-Sandholm *et al.*, 2002). Many strains do not survive the drying process, have a long lag phase in recovering from the process, and do not remain viable during extended storage at room-temperature. Several methods have been described to stabilize probiotic cells (Mattila-Sandholm *et al.*, 2002). Fooks *et al.* (1999) suggested the use of prebiotics, defined as "non-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon, especially *Lactobacillus* and *Bifidobacterium* spp." (Gibson and Roberfroid 1995), as stabilizing agents. The survival rate of the culture during spray drying and subsequent storage depends upon a
number of factors, including species and strain of culture, drying conditions, inoculum/medium used, pre-adaptation of the culture to acquire resistance to processing conditions and the use of protective agents (Teixeira et al., 1995; Bielecka and Majkowska 2000; Conrad et al., 2000; Gardiner et al., 2000; Desmond et al., 2001). Gardiner et al., (2000) have reported that the viability of the probiotic *L. paracasei* NFBC 338 following spray drying is poor and inversely related to storage temperature. The temperature of outlet-air also affects the survival of the cell during spray drying. Kim and Bhowmik, (1990) observed survivals of 2% and 0.01 % at outlet-air temperatures of 65 and 90°C, respectively. Metwally et al., (1989) found survivals of 47% and 9% at outlet-air temperatures of 71 and 89°C, respectively. In a study, it was reported that the use of a carrier material composed of poly vinyl pyrolidone and dextran to encapsulate *Enterococcus faecium* during spray-drying resulted in an increase in viability of up to 15% following 4 months of storage at room-temperature when compared with a non-encapsulated control (Millqvist-Fureby et al., 2000). However, microencapsulation with starch proved to be unsuitable for use as an encapsulating material for a probiotic *Bifidobacterium* strain during spray-drying, storage and acid stress conditions (O’Riordan et al., 2001). It was shown that survival of yoghurt cultures and other bacteria during drying was affected by many factors, such as composition of the medium, growth phase (Teixeira et al., 1994), biomass concentration (Bozoglu et al., 1987), and protective factors. Although, Desmond et al., (2001) improved the viability of probiotic cultures during spray drying by environmental adaptation to heat and salt stress prior to drying, the stability of the dried culture during storage was not markedly enhanced. The addition of thermo-protectants, such as trehalose (Conrad et al., 2000), casein or WPC (Kelleyet
and / or adonitol (Selmer-Olsen et al., 1999), growth-promoting factors, including prebiotics such as starch, oligosaccharides (Mitsuoka, 1992) and granular starch (Crittenden et al., 2001) have been employed to improve culture viability of probiotics during drying, storage and/or gastric transit. The addition of various protective compounds to probiotic cultures can improve their viability during manufacture for example glucose to energize cells on exposure to acid (Corcoran et al., 2005) and cryo-protectants such as inulin to improve survivability during freeze drying (Carvalhoet al., 2004). The use of stabilizers has been reported to reduce the loss of cell viability during freeze-drying (Valdez Font de et al., 1983; Orindorff and MacKenzie, 1973). In order to minimize viability losses incurred during the drying process, several investigators have evaluated various drying methods. In a recent study by Lianet al., (2002), four strains of Bifidobacterium was successfully spray-dried with gum acacia and other carrier materials such as gelatin and soluble starch. The authors concluded that the survival of these probiotics varied with strains and is highly dependent on the carriers used. Desmond et al., (2002) have reported that the use of gum acacia in the suspending medium prior to spray drying provide a technological benefit in order to preserve the viability of dried L. paracasei NFBC 338 during storage at elevated temperatures and potentially during gastric transit. While in another study Desmond et al., (2004) showed that probiotic cultures that over-express the heat shock proteins GroESL and have demonstrated improved performance under a variety of conditions including heat, spray drying and exposure to gastric acid have been generated. No reports are available on the effect of packaging material on the storage stability, but Kumar and Mishra (2004b) studied the effect of packaging material viz.; HDPP (high density poly propylene) and pouches of
lamine of PE and aluminum (PE/Al) on storage stability of mango soy fortified yoghurt powder and reported that a laminate of PE/Al is best packaging material than HDPP in all respect when stored at 38±1°C at 95% relative humidity.

2.9 Microwaves for Yoghurt Drying

Microwave used in food industry for heating are the Industrial, Scientific and Medical (ISM) frequencies 2.450 MHz or 915 MHz, corresponding to 12 or 35 cm in wave length. Microwaves are generated by magnetron that converts electrical energy (60 Hz) into an electromagnetic field with centers of positive and negative charges. Majority of food contains water. The molecular structure of water consists of a negatively charged oxygen atom and positively charged hydrogen atoms and it forms an electrical dipole. When microwave is applied to a food, dipoles in water attempts to orient themselves to the field and rapidly oscillating electrical field changes from positive to negative and back again several million times per second, the dipole attempts to follow and these rapid reversals create frictional heat. The increase in temperature of water molecules heats surrounding components (Ohlsson and Bengtsson, 2002). Microwave were reflected by metals, transmitted by electrically neutrals such as glass, most plastics, ceramics, paper and absorbed by electrically charged materials like several food constituents including water (Mullin, 1995).

Although microwave was introduced in 1960’s, only at the beginning of 1980’s, the possibilities of applying microwave energy were widely recognized. The advantages evident, such as faster operation, energy saving, higher product quality (retention of nutritional and sensory properties) as an application like enzyme inactivation,
pasteurization, tempering and drying of food products (Fito et al., 2005). Microwave had been successfully used in the food and dairy industries in processing of fruits, vegetables, cereals, meats, butter, cheese, yoghurt and ice cream mixes to inactivate number of microbes, enzymes and bacteriophages (Young and Jolly, 1990; Schiffman, 1992).

Microwave had been successfully in dairy industry used for pasteurization of milk, by exposing loose or packed milk to microwave which retained better nutritional properties in comparison to the present technologies can be obtained due to the rapid, uniform heating and because of lack of hot contact surfaces (Villamiel et al., 1996; Fito et al., 2005; Valero et al., 2000). It can also be used for milk sterilization (Assinder, 1971), thawing of frozen foods such as ice cream and butter (Young and Jolly, 1990) and cooking of cheese (Hussain et al., 1980).

Suk-Shin-Kim and Bhowmik (1994) studied the moisture sorption isotherms of concentrated yoghurt and microwave vacuum dried yoghurt powder reported that yoghurt showed higher sorption energy levels than those of concentrated yoghurt at moisture content of 0.025 kg water/kg of solid or higher. Three yoghurt powders processed from the concentrated yoghurt showed very similar heat curves. There is no literature available on used of microwave in probiotic yoghurt processing.

2.10 Modified Atmosphere Packaging (MAP)

MAP is the enclosure of food in a package, inside which the atmosphere is modified with respect to carbon dioxide, oxygen, nitrogen, water vapour and trace gases. This modification is generally achieved using one of the two processes such as gas flush packaging or vacuum packaging (Farber, 1991). According to UK institute of packaging,
packaging can be best defined as a coordinated system of preparing goods for transport destruction, storage, retailing and end use a means of ensuring safe delivery to the ultimate consumer in sound condition and minimum cost. The packaging concept is determined by the demand of the consumer and the product, new technological developments environmental awareness and changes in the consumer market force for packaging technologists to consider an increasing number of factors when designing a package (Goyal and Tanweer, 2006)

MAP, as the name implies is one in which the normal composition of air is changed or modified within a high barrier package. The technique of MAP has been developed partly to retard the activity and growth of bacteria and fungi and partly to delay biochemical reactants such as oxidation. MAP is extensively used to preserve a wide array of food products (Farber, 1991). The technology of packaging products in modified atmosphere is the most advanced food preserving technique with many advantages (Floros et al., 2000). Enfors and Molin (1980) reported that the gram negative bacteria were more sensitive to CO₂ while, the growth rates of LAB were much less affected. Various inhibition actions of CO₂ on bacterial cell have been suggested (Farber, 1991; Davis, 1995) that displacement of oxygen, penetration of cell membrane leading to changes in intracellular pH, direct changes brought in the physico-chemical properties of cell proteins, direct inhibition of enzymes or decrease in the enzyme activity and alteration of cell membrane, effect on nutrients uptake and absorption. The nitrogen (N₂) another widely used MAP gas, is considered as an inert and filler gas and replaces the oxygen in the pack thus delay the oxidative rancidity of the product and inhibits the growth of aerobic microbes indirectly (Sahoo and Anjaneyalu, 1995).
Bacillus anthracis could be inactivated by using MAP (Sivertsvik et al., 2002). The modified atmosphere composition has a marked impact on the growth of spoilage micro-organisms as well as on pathogens. The anti-microbial effect of CO₂ on microorganisms has been intensively documented. However, it has been shown recently that only CO₂ levels well above 20% significantly affect the growth of psychrotrophic pathogens that are relevant to modified atmosphere packaging (Bennik et al., 1995). Fedio et al., (1994) investigated the effect of MAP on the growth of microorganisms in cottage cheese and reported that samples inoculated with Listeria showed growth in packages containing air and 100% nitrogen but not in packages containing elevated carbon dioxide levels. The 100% CO₂ environment inhibited the growth of many microorganisms and extended the shelf life of cottage cheese to 60 days.

Karagul-Yaceer et al., (2001) reported that carbonation of yoghurt declined E. coli and L. monocytogenes counts without significantly affecting the growth of typical yoghurt bacterial strains and probiotic organisms in yoghurt. Gas flushing with CO₂ or N₂ was a viable alternate preservation method to extend the shelf-life of plain and fruit yoghurt (Tamime and Deeth, 1980; Fairbian and Law, 1986; Ogden and Inventor, 1997). It is a cheap, safe and apparently does not have any negative impact on the quality of yoghurt (Fairbian and Law, 1986). The effect of O₂ toxicity on survival of probiotic strains of Lactobacillus and Bifidobacterium spp. had been studied widely by Talwalkar and Kailasapathy, (2004).