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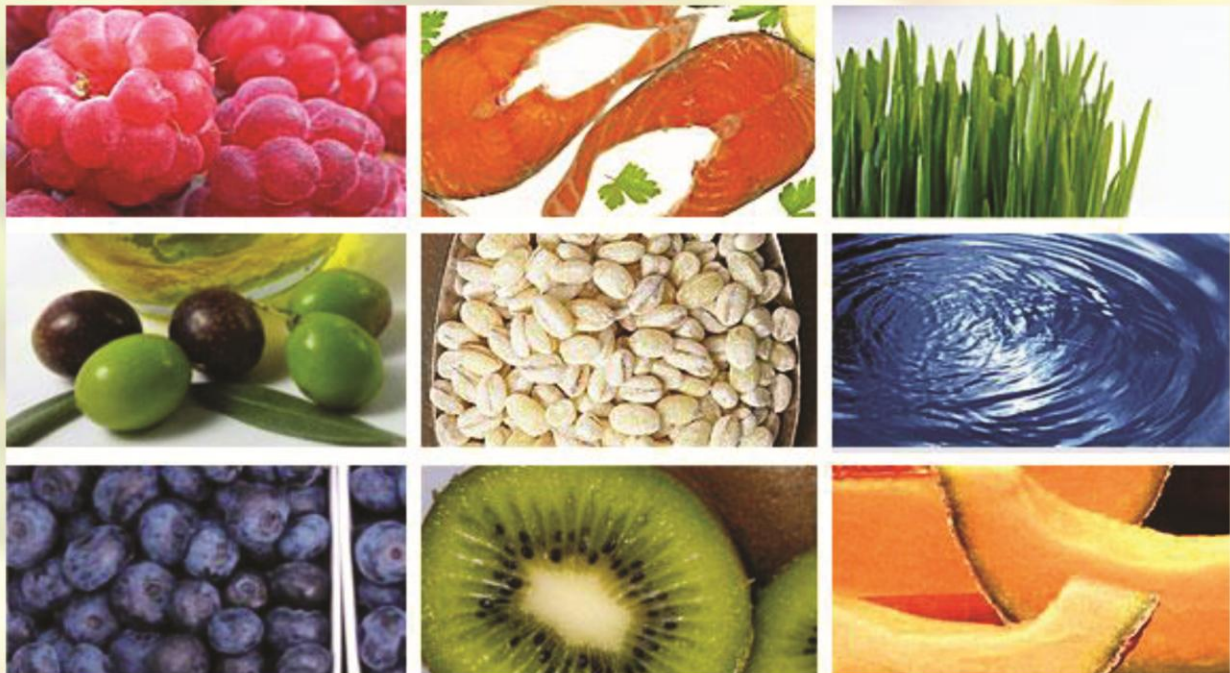
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## YOGHURT IS EXCELLENT VEHICLE FOR TRAVELLING PROBIOTICS TO PUBLIC HEALTH

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### ABSTRACT

Yogurt is a product of the lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. In some countries less traditional microorganisms, such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii* ssp. *lactis*, are sometimes mixed with the starter culture. Yoghurt is a fermented milk product with custard like consistency. The popularity of yoghurt has increased significantly in the last few decades because of incorporation of probiotics microorganism into the product that gives an extra nutritional physiological value. The most popular probiotics bacteria that are added to the yoghurt are *lactobacillus acidophilus* and *bifidobacterium bifidum* and the product called as bio- yoghurt. To achieve the therapeutic value, it is suggested that the bio yoghurt should be consumed for more than 100g/d, containing viable probiotic cells of more than  $10^6$ - $10^7$  cfu/ml.

### INTRODUCTION

The concept of probiotics was used in the early 1900s; however, the term was coined in 1965 by Lilly & Stillwel (1965). Probiotic cultures are live microbials that have beneficial effects on human health (Salimen et al., 1998). The most commonly added cultures include LA and BB (Murti et al., 1993; Lourens - Hattingh & Viljoen, 2001; Torre et al., 2003). It is a very beneficial product for some individuals who are lactose-intolerant and have problems in digesting milk products (Vijayvendra & Gupta, 1992; Pereg et al., 2005). They provide proven health benefits including lowering cholesterol (Taylor & Williams, 1998; Chagarovskii & Zholkevskaya, 2003), aiding digestion and protection against intestinal infection and colon cancer (Sanders, 1999). The introduction of fermented milk products such as cheeses and yogurts in to the diet of man is thought to date back to the dawn of the civilization (Mckinley, 2005). Consumption of fermented-milk products is associated with several types of human health benefits partly because of their content of lactic acid bacteria. Several experimental observations have indicated a potential effect of lactic acid bacteria (LAB) against the development of colon tumors (Wollowski et al. 2001). Recently, the role of fermented milks containing lactic acid bacteria (LAB), such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus thermophilus*, has been studied (Saikali et al. 2004). A wide range of other health benefits, including improved lactose digestion, diarrhea prevention, immune system modulation and serum cholesterol reduction, have been ascribed to fermented milk consumption. Milk is a

complete food, gifted by God to human being. Fluid milk is not only nature's food for a new born infant, but also a source for a whole range of dairy products consumed by mankind. Fermentation is one of the oldest methods practised by human beings for the transformation of milk into products with an extended shelf life. The exact origin of the making of fermented milks is difficult to establish, but it could date from being food gatherer to food producer.

The introduction of fermented milk products into the diet of man is thought to date back to the dawn of civilization, as reference is made to them in both the bible and sacred books of Hinduism. According to persian, Abraham owed his fecundity and longevity to yoghurt and, earlier, emperor Francisi of France was said to have been cured of a debilitating illness by consuming yoghurt made from goat's milk (Tamime and Robinson, 1985). Fermented milk is a good source of protein, calcium, phosphorus, and B vitamins, including vitamin B<sub>12</sub>. In addition to their nutritional value, it has therapeutic and preservative potential. Fermented dairy offers a natural way to deliver what scientists call probiotics (beneficial bacteria such as *lactobacilli* and *bifidobacteria* to the intestines).

The concept of probiotics evolved around 1900, when Nobel Prize winning Elie Metchnikoff hypothesized that the long, healthy lives of Bulgarian peasants were the result of their consumption of fermented milk products and later he was convinced that yoghurt contained the organisms necessary to protect the intestine from damaging effects of other harmful bacteria. According to the currently

adopted definition by FAO/WHO, probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host"(FAO/WHO Joint report, 2001). Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures, such as in yogurt, soy yogurt, or as dietary supplements. Various fermented milk product like sour cream, cheese and fermented vegetables like sauerkraut are also considered as potential sources of probiotics. Probiotics are also available in the form of tablets, capsules and powder. (Pravinder Kaur and Satyanarayana, 2004). The trial for production of fresh cheese incorporating probiotics *L.acidophilus* group lactic acid bacteria was shown as a promising food for a delivery system of probiotic bacteria (Masuda *et al.* 2005).

Yogurt in different forms with diverse local names is made throughout the world (Tarakci and Küçüköner, 2003). The use of yogurt dates back many centuries, although there is no accurate record of the date when it was first made. According to legend, yogurt was first made by the ancient Turkish people in Asia. The uniqueness of yogurt is attributable to the symbiotic fermentation involved in its manufacturing. The composition of yogurt is dependent on the type and source of milk and a range of seasonal factors. For example: whole milk or skimmed milk, season, lactation period and the feeding mode. It is also significantly influenced by manufacturing conditions (such as temperature and duration and equipment utilized) and on the presence of other ingredients such as powdered milk or condensed milk, (Blance, 1986). Yogurt is derived from Turkish word *Jugurt* describing any fermented food with acidic taste. Its manufacture involves the use of specific symbiotic/mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Kon, 1959). Cultured (fermented) dairy foods are milk products that result from the fermentation of milk or its products by starter cultures (selected specific microorganism) that produced lactic acid under controlled conditions. There are variety of cultured dairy foods differing in flavor and consistency such as acidophilus milk, Bulgarian buttermilk, cultured buttermilk, cultured or sour cream, Kefir, Koumiss and yoghurt (Kosikowski & Mistry 1997). Fermentation of milk results in partial breakdown and better absorption of carbohydrates (lactose) and fat (Hitchins & Macdonough 1989) of the milk. Yoghurt is an easily digested product of milk.

Yoghurt is one of the best-known of the foods that contain probiotics. Yoghurt is defined by the Codex Alimentarius of 1992 as a coagulated milk product that results from the fermentation of lactic acid in milk by *streptococcus thermophilus* and *lactobacillus bulgaricus* (Bourlioux and Pochart 1988). As other lactic acid bacteria have also many beneficial effects on health, lactic acid bacteria (LAB) are combined with *streptococcus thermophilus* and *lactobacillus bulgaricus* for preparation of yoghurt and other fermented foods in the recent years In the finished product the LAB must be alive, in substantial

amounts. LAB has been used for thousands of years to produce fermented food and milk products. Fermented food contains a variety of fermenting microorganisms belonging to various genera and species, all of which produce lactic acid (Simin and Woel –kyu *et al.* 2000).

The term 'yoghurt like product' is defined as alternative culture yoghurt (i.e., when *L. bulgaricus* is substituted by other *Lactobacillus* species for the fermentation of milk or yoghurt containing probiotics bacteria (when probiotics bacteria are added to the yoghurt or alternative cultures) (Francisco *et al.* 2005).

Yogurt is a product of the lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. In some countries less traditional microorganisms, such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii* ssp. *lactis*, are sometimes mixed with the starter culture (McKinley, 2005). Yoghurt is a fermented milk product with custard like consistency.

Yogurt's nutritional profile has a similar composition to the milk from which it is made but varies somewhat, if fruit, cereal or other components are added. Yogurt is an excellent source of protein, calcium, phosphorus, riboflavin (vitamin B<sub>2</sub>), thiamin (vitamin B<sub>1</sub>) and Vitamin B<sub>12</sub>, and a valuable source of folate, niacin, magnesium and zinc. The protein it provides is of high biological value, and the vitamins and minerals found in milk and dairy foods including yogurt are bioavailable. Yogurt, particularly the low-fat varieties, provide an array of important nutrients in significant amounts in relation to their energy and fat content, making them a nutrient-dense food. Eating dairy products, such as yogurt, helps to improve the overall quality of the diet and increases the chances of achieving nutritional recommendations. (Mckinley, 2005). Yogurts may be spoonable or drinkable, and may be considered dietary supplements for infant consumption. So they cross the line between dietary supplements, medical foods, and conventional foods (Katz, F. 2001). Although fermented milk products such as yogurts were originally developed simply as a means of preserving the nutrients in milk, it was soon discovered that, by fermenting with different microorganisms, an opportunity existed to develop a wide range of products with different flavours, textures, consistencies and more recently, health attributes (Mckinley, 2005).

## **DIFFERENT TYPES OF YOGHURT AND PROBIOTICS DAIRY PRODUCTS**

### **1.1. DIFFERENT TYPES OF YOGHURT**

Commercial yoghurt is divided into three main categories, i.e., fruits flavoured yoghurt, frozen yoghurt or yoghurt ice-cream and dried yoghurt. They are discussed as following ways:



### **1.1.1. FLAVOURED YOGHURT WITH FRUIT/ FLAVOURED SYRUP**

Flavored yogurts are made by adding fruit concentrates or flavored syrups to cultured milk before or after incubation (Keating and White, 1990). Fruit yogurt, a popular type of yogurt is liked by masses and is known as fruit stirred yogurt. Yogurt prepared by adding seasonal fruits are very attractive. Fruit stirred yogurt is popular among masses and particularly children who dislike the flavour of plain yogurt. This modification has made the yogurt flavor attractive for them. Addition of fruit makes the yogurt more delicious. The product contains both the nutritive effect of yogurt and refreshing taste of fruit. Fruit stirred yogurt has more sweetness and pleasing flavor (Hursit and Temiz, 1999).

Attempts have been made to fortify yoghurt with fruits and additives which included bilberries, pineapple, black currants (Arnold 1963), banana (Schulz *et al.* 1965), vegetables and fruits (Fekete and Buchi 1972) and sweetened fruit yoghurt (Singh 1983).

Patil *et al.* (2009) reported that Guava pulp at 5% could be very well accommodated in yoghurt to produce good blend of mild guava flavor with acidic taste of finished product. Guava fruit is therefore a very good additive to yoghurt, which not only improves the acceptability of the product but also enhances its vitamin C content.

Torreggiani *et al.* (1994) in their study reported addition of osmohydrofrozen fruits to yoghurt. They used osmohydrofrozen apricot and peach cubes for producing fruit yoghurt. It prevented whey separation because of controlled amount of moisture absorption by semi dried fruit cubes.

Bakirci & Kavaz (2008) revealed that addition of fruit and sugar at different levels did not have any marked effect on the apparent viscosity, viable counts of *S. salivarius* ssp. *thermophilus*, *L. acidophilus* and bifidobacteria, and scores of consistency (by spoon and mouth) or aroma. The values of syneresis, titratable acidity and pH, as well as the scores for appearance and overall acceptability were significantly affected by the added fruit and sugars. On the other hand, the values of apparent viscosity, the counts of *L. acidophilus* and bifidobacteria, and all sensory evaluations performed by panelists were not significantly affected by storage time, except for the counts of *S. salivarius* ssp. *thermophilus*. In the sensory evaluation, sample containing 10% banana puree and 5% sugar, was preferred more than control by panelists. It was concluded that a natural fruit such as banana can be used in the manufacture of bio-yogurt made with a commercial probiotic starter culture.

Tamime (2006) revealed that a post-fermentation heat treatment of stirred and fruit flavoured yoghurt helps to prolong the shelf-life of the product, since the application of heat inactivated the starter culture bacteria (e.g. *Streptococcus thermophilus* and *Lactobacillus delbrueckii*

*subp. bulgaricus*) and their enzymes, as well as other contaminants, such as yeasts and molds.

### **1.1.2. FROZEN YOGHURT OR YOGHURT ICE CREAM**

Frozen yoghurt is classified into three main categories, soft, hard or mousse. These products resemble ice-cream in their physical state, and they are characterized simply as having the sharp, acidic taste of yoghurt combined with the coldness of ice-cream.

Venkateshaiah *et al.* (1997) revealed that yoghurt ice-cream prepared by replacement of skimmed milk powder with whey solids had an overrun of 77%, melting time of 5.4 min and a starter culture count of  $7.2 \times 10^8$  cfu/gm; however the sensory properties of the product were further enhanced by blending the yoghurt with an ice-cream base mix at a ratio of 40:60; the viable cell count marginally decreased after storage at -20°C for 10 days.

Ozdwmir *et al.* (1999) revealed that storage of frozen yoghurt for longer than 2 months affected the sensory properties of the product. The use of capsule-forming strains of lactic acid bacteria in making frozen yoghurt increased the apparent viscosity and over-run, reduced the rate of melting, made the culture more resistant to heat-shock and the product was more acceptable than a parallel product made with non-capsule forming starter culture.

Mostafa *et al.* (2001) studied that the cultured buttermilk, fermented skimmed milk and replacement of the fat with reduced cholesterol butter oil were used successfully during the manufacture of yoghurt ice-cream. The product that contained cultured buttermilk had more volatile fatty acids and acetaldehyde, increased overrun and had good flavor, body and texture when compared with the control frozen yoghurt.

Miscellaneous factors that have been reported to affect the quality of yoghurt ice cream are: the level of sugar and fruit concentration (Güven & Karaca 2002); and replacing sucrose with stevia sweetener by 75 % (Salem & Massoud, 2003).

### **1.1.3. DRIED YOGHURT/POWDERED YOGHURT**

The primary objective of manufacturing powdered yoghurt is to produce a product that is stable during prolonged storage and readily utilizable. Nevertheless, dried yoghurt is widely used in the food sector in the manufacture of sauces, soups, baked goods and baby foods (Tamime, 2003). The addition of hydrocolloids during the manufacture of spray-dried yoghurt has improved the following characteristics of the product when compared with ordinary dried yoghurt: retention of volatile compounds such as acetaldehyde; and solubility and dispersion of the powder during reconstitution (Ramirez-Figueroa *et al.* 2002; Crofsky *et al.* 2004).

#### 1.1.4. FERMENTED MILK AND BEVERAGES

A wide range of fermented milk products are made in different countries, but the classical example is yoghurt, which is manufactured as either set, stirred and drinking types, with the products being flavoured by adding fruit preparations or fruit essences plus coloring matter (Tamime *et al.* 1995; Tamime & Robinson, 1999; Bottazzi, 2002)

Cultured butter milk is made by the microbial fermentation of pasteurized whole milk or skimmed milk. Probiotic cultured buttermilk contains different microorganisms beside the lactic starter cultures, such as *Lb. rhamnosus GG* (piima in Finland, hapupiim in Estonia, and syrnnet melk in Norway), *Lb. Rhamnosus 271* (viilijuoma in Finland), *Lactobacillus reuteri* (piima in Finland), *Lactobacillus casei 421* (piima in Finland) and with *Lb. acidophilus* alone or together with *Bifidobacterium spp.* (fil in Sweden, Piima in Finland and buttermilk in Egypt; El-Shafei, 2003)

Kefir is a carbonated milk product made by using a complex mixture of microorganism known as Kefir grains. Typically, these include *Lac. Lactis subsp. Cremoris*, *Lac. lactis subsp. Lactis*, *Lactobacillus Kefir*, *Lactobacillus Kefiranofaciens*, *Lactobacillus brevis*, *Lb. acidophilus*, *Leuconostoc spp.*, *Acetobacter spp.*, lactose fermenting yeasts (*Kluyveromyces spp.*) and lactose-negative yeasts (*Saccharomyces spp.*, *Candida spp.*) (Marshall 1987; Tamime & Marshall 1997). Commercial Kefir starter cultures are available in different blends of thermophillic and mesophillic lactic acid bacteria and yeast cultures, which allows the flavor profiles of the product to be based on consumer acceptability. Recently, bio Kefir has been marketed in Poland containing *Bifidobacterium spp.* and/or *Lb. acidophilus* (Muir *et al.* 1999).

#### 1.1.5. CHEESES

Various cheese varieties have been successfully used as a carrier of probiotics microorganisms. Some cheese may be particularly suitable for the delivery of probiotic relative to fermented milks such as yoghurt, because of lower acidity and the existence of a complex cheese matrix of protein and fat that will provide protection to probiotic microorganisms during their passage through the gastrointestinal tract (Stanton *et al.* 1998; Donnelly, 2003).

Probiotic bacteria may be introduced into cheese as adjunct cultures along with the lactic starter cultures (Stanton *et al.* 1998; McBrreaty *et al.* 2001; Perko *et al.* 2002). In a variation of this method, probiotic Cheddar cheese was manufactured by standardizing the cheesemilk with cream that had been fermented with *Bifidobacterium infantis* (Daigle *et al.* 1999). In a study by Dinaker & Mistry, 1994, two preparations of *Bifidobacteria* were used; one was *B. bifidum* preparation in which live bacteria were immobilized by forming gelled beads in carrageenan; these beads were then freeze-dried. The second preparation was a commercial powder preparation containing *B. bifidum*. These preparations were added separately to the cheese curd at milling, such that the viable counts of *bifidobacteria* in the cheese were  $10^6$  cfu/gm.

In Finland *Lb. acidophilus* and *Bifidobacterium spp.* were used to produce a low-fat cheese (Ryhanen *et al.* 2001). These organisms apparently produce bioactive peptides with anti-hypertensive properties during maturation of the cheese, thus adding to the nutritional and probiotic values of the product. The inclusion of probiotic cultures, such as *Lb. rhamnosus GG*, in cheese may have an impact on dental caries (Abou-Dawood, 2002).

A spray-dried probiotic milk powder containing *Lactobacillus paracasei NFBC 338* (Rifir) was produced with a probiotic survival rate of 84.5%, and this was used in the manufacture of cheddar cheese as an adjunct culture (Gardiner *et al.* 2002). The initial *Lb. paracasei* count of  $2 \times 10^7$  cfu/gm increased to  $3.3 \times 10^7$  cfu/gm in 3 months. *Lb. Gasseri* grows slowly in milk, so it is difficult to use it independently for cheese making; however when used in combination with *S. thermophilus*, it maintained viability in a semi-hard cheese (Gardiner *et al.* 2002).

#### 1.1.6. PROBIOTICS ICE CREAM

Probiotics ice cream is made by fermenting a standard ice cream mix with *Lb. acidophilus* and *Bifidobacterium bifidum* cultures and then freezing the mix in a batch freezer. It was demonstrated that probiotic ice cream can be a suitable vehicle for delivering beneficial microorganisms such as *L. acidophilus* and *B. bifidum* to consumers. The bacteria can be grown to high numbers in the cream mix and remain viable during storage (Sharareh Hekmat and Donald, 1992).

A mixture of human-derived probiotic strains of *Lactobacillus acidophilus*, *L. agilis* and *L. rhamnosus* was used as a probiotic culture in ice cream manufacture. Viability and survival of these probiotic cultures were investigated in two different ice cream formulations. Ice cream with sucrose and ice cream with aspartame were prepared and each of these was divided into two subgroups: one the one with addition of the probiotic culture and one with milk fermented by the same probiotic culture. Ice cream samples were stored at  $-20^\circ\text{C}$  for 6 months and the survival rate of cultures were determined at monthly intervals. Probiotic cultures underwent tests for resistance to bile salts, antibiotics, acidic conditions; they were found to be highly resistant to such challenges. Chemical analysis of ice cream samples, such as determination of acidity, pH and solid matter, was also performed. The probiotic cultures remained unchanged in ice cream stored for up to 6 months regardless of the sweeteners used. Using probiotic cultures in ice cream mixes did not alter the characteristics of the product. (Gülden *et al.*, 2006).

Properly selected strains, such as *Lactobacillus johnsonii* La 1, survive the relatively high sugar content of ice-cream as well as the sub-lethal injuries caused by freezing. Counts of  $10^7$  cfu  $\text{g}^{-1}$  were maintained for 10 weeks (Kebary *et al.* 1998; Kebary *et al.* 2004; El Shazly *et al.* 2004; El Tahra *et al.* 2004a, 2004 b; Hamed *et al.* 2004; Rao & Prakash, 2004) or 8 months of storage (Alamprese *et al.* 2002). Some strains do not resist the freezing and churning that occurs during ice-cream manufacture (Hagen

& Narvhus, 1999; Haynes & Playne, 2002), but others such as *B. longum* and *B. infantis* were able to survive these processes and storage up to 11 weeks (Davidson *et al.* 2000) or 52 weeks (Haynes and playne, 2002), and are not influenced by the fat content of the product.

## **1.2. HEALTH BENEFITS OF YOGHURT IN LACTOSE INTOLERANCE**

Lactose is the main carbohydrates of milk and dairy products. Lactose maldigestion is difficulty in digesting the disaccharide, lactose due to insufficient amounts of the enzyme lactase.

Research has shown that lactose in yoghurt is tolerated better by individuals with hypolactasia than an equivalent quantity in milk (Gallagher *et al.* 1974; Kolars *et al.* 1984.; McDonagh *et al.* 1987; Dewit *et al.* 1988; Marteau *et al.* 1990 and Rosado *et al.*1992).  $\beta$ -galactosidase (lactase) is an intracellular enzyme and so will remain active as long as the cell wall of the bacteria( whether viable or nonviable ) remains intact. This theory is, however, confounded by the results of a study that found no difference in digestion and tolerance to lactose while ingestion of fermented food products that had a fourfold difference in their  $\beta$ - galactosidase activity. These results suggest that factors in addition to lactase activity may be contributing to the improved lactose tolerance associated with yoghurt. For example, the different viscosity of yoghurt compared to milk may result in slower gastric emptying and thus a longer transit through the gastrointestinal tract, which, in turn, may improve the absorption and reduce the lactose load in the colon (Vesa *et al.* 1996).

The most obvious explanation is that microorganism in the yoghurt continue to metabolise the lactose even after ingestion, so that the residual lactose content reaching the small intestine is too low to cause an adverse reaction. Certainly some lactic organisms would be tolerant of the acidity of the human stomach e.g., *Lactobacillus acidophilus*, and the presence of such bacteria in at least some starter cultures is a reasonable surmise. Even some strains of *L. bulgaricus* are tolerant of the low pH encountered, and so it is feasible to suggest that some breakdown of lactose does continue in the stomach, and even into the intestine. One further effect that may be relevant in this context is that yoghurt is already coagulated prior to entering the stomach, while liquid milk is clotted by the acid/enzymes in the body (Tamime & Robinson, 1999)

Kolars *et al.* (1984) used a series of breath hydrogen tests as well as a subjective assessment to ascertain whether subjects who were identified as lactose – intolerant digested and absorbed lactose in yoghurt better than lactose in milk. The area under the curve for breath hydrogen was smaller after yoghurt consumption of milk or lactose in water. Subjective assessment by the subjects in the study also indicated that lactose in yoghurt was better tolerated than the same amount of lactose from milk or in water.

Using breath hydrogen measurement, Savianno *et al.* (1984) also investigated the effects of 3 varieties of

cultured milk products on the digestion of lactose by 9 lactase –deficient human subjects. When yoghurt, cultured milk (buttermilk), and sweet acidophilus milk were compared, yoghurt had the most beneficial effect on lactose digestion. The study revealed that Lactose activity an the number of surviving LAB were significantly reduced when the yoghurt was pasteurized.

### **1.2.1. EFFECT ON WEIGHT CONTROL AND LOWERING CHOLESTROL**

Skinner *et al.* (2003) in a longitudinal study of infants who were followed until they were 8 years old found that a higher dietary calcium intake from calcium-rich foods like milk, cheese and yoghurt was associated with a lower percentage of body fat. In a study by Zema *et al.* (2003) 34 healthy but obese woman received 1100 mg calcium /days(in the form of three serving of fat-free yoghurt) or 500 mg of supplemented calcium /day ,both groups following a calorie –controlled diet. After 12 weeks ,the researchers found that the yoghurt group lost 22% more weight ,61 % more body fat and 81% more trunkal fat than the other group.

Cholesterol lowering as a potential beneficial effect of probiotics yoghurt has gained much interest. Several *L.acidophilus* strains of human origin are able to remove cholesterol from culture media during growth in the presense of bile. Anderson and Gilliland (1999) hypothesized that every 1% reduction in serum cholesterol concentration is associated with an estimated 2-3% reduction in risk of coronary heart disease. A regular intake of fermented milk containing an appropriate strain of *L.acidophilus* has the potential lower risk of coronary heart disease by 6-10% .The daily intake of 375 g *L.acidophilus* –yoghurt and tablet with lyophilized *L. acidophilus* caused a decrease of total cholesterol in male volunteers by 4.4% and 5.3% respectively.(Lin *et al.* 1989; Schaafsma *et al.*1988).

The long term daily consumption of 300 g of yoghurt fermented by traditional starter cultures and supplemented with *L. acidophilus* and *B. longum* did not lower the total and LDL cholesterol in 29 healthy women. The increase of the HDL cholesterol may be an effect of the long- term daily consumption of yoghurt (21weeks) and lead to an improvement of the LDL/HDL ratio (Kiebling *et al.* 2002).

Regular consumption of both probiotic and conventional yoghurt for 4 weeks had a positive effect on the lipid profile of healthy women (Fabian *et al.* 2006).

Yoghurt containing two probiotics bacteria strains *L. acidophilus* and *B.lactis* had a cholesterol lowering effect in hypercholesterolemic subjects (Asal Ataie-Jafari *et al.* 2009).

### **1.2.2. EFFECT ON DIARRHOEL DISEASES**

Best documented evidence exists for the shortening of rotavirus diarrhea in children by selected probiotic strains such as *L.rhamnosus GG* (Guandalini *et al.* 2000).



A recent Meta –analysis of randomized, controlled studies by (Van Neil *et al.* 2002) found that therapy using *Lactobacillus* strains offered a safe and effective means of treating acute infectious diarrhoea in children .Both the duration and frequency of diarrhoeal episodes were reduced when compared with those in control subjects. The benefits of *Lactobacillus* therapy was seen in diarrhoeal diseases caused by various pathogens. The effect of supplementing formula with *B.bifidum* and *S thermophilus* on preventing the onset of acute viral diarrhea in infants was examined in a double-blind, Placebo-controlled trial (Saavedra *et al.* 1994). A recent meta analysis evaluated the ability of several different probiotics LAB species to prevent antibiotic –associated dairrhoea. ( D’souza *et al.* 2002)

Prevention and treatment of antibiotic –associated diarrhea appears to be feasible for selected probiotic strains including *L.acidophilus*, *L.delbrueckii spp.Bulgaricus* and *B.longum* with good evidence for *Enterococcus faecium SF68*, *L.rhamnosus GG* and *Sacchromyces boulardii* (Marteau 2001 ,2002).

Supplementation of infant formulas with *bifidobacterium lactis* and *S. thermophilus* has been reported to be protective against nosocomical diarrhea in infants (Saavedra *et al.* 1994).

Research has also shown that several strains of probiotics are helpful in the prevention and treatment of antibiotic-associated diarrhoea (*Lactobacillus GG* and *Saccharomyces boulardii*) (Jones, 2010).

Four different diets were used in the study by De Mattos *et al.* 2009: diet 1, yogurt-based formula; diet 2, soy-based formula; diet 3, hydrolyzed protein-based formula; and diet 4, amino acid-based formula .The data showed that children fed yogurt-based diet (diet 1) or the amino acid-based diet (diet 4) had a significant reduction in stool output and in the duration of diarrhea.

The use of an inexpensive and worldwide-available yogurt-based diet is recommended as the first choice for the nutritional management of mild to moderate (persistent diarrhea) PD. For the few complicated PD cases, when available, a more complex amino acid-based diet should be reserved for the nutritional management of these unresponsive and severe presentations.

### 1.2.3. EFFECT ON COLON CANCERS

Wollowski, *et al.* (1999) investigated the protective effect of several strains of LAB, traditionally used for milk fermentation, against 1, 2-dimethylhydrazine (DMH)-induced colon carcinogenesis in rats. Oral treatment with *L. bulgaricus* through a fermented milk for 4 day protected against DMH –induced DNA damage in the colon.

Using a colon carcinoma cell structure system, Ganjam *et al.* 1997 isolated a yoghurt fraction that decreased cell proliferation, as ascertained with the use of thymidine incorporation. Cell proliferation was not inhibited in response to a similarly isolated milk fraction or to a lactic acid.

Kampman *et al.* (1994) reported an inverse relationship though non-significant, between colonic

ademonas and yoghurt consumption. A case control study of Boutron *et al.* 1996 showed a significant inverse relationship between consumption of moderate amount of yoghurt and the risk of colonic adenomas in both women and men.

### 1.2.4. EFFECT ON INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is a term used for certain chronic immune –mediated conditions of the intestinal tract. Bourrel *et al.* 2002 reported that, when inflamed intestinal mucosa from a group of crohn’s disease patients was cocultured in the presence of *L. bulgaricus* or *L.caseii*, expression and release of TNF- $\alpha$  by intraepithelial lymphocytes were reduced. A recent randomized controlled study examined the effect of supplementation with 100 ml *bifidobacteria* –fermented milk daily for 1year as a dietary adjunct in the treatment of ulcerative colitis. Exacerbation of symptoms was seen in 27% of the *bifidobacteria* fermented milk group compared to 90% of controls. The probiotics supplementation was successful in maintaining remission and had possible preventive effects on the relapse of ulcerative colitis. (Ishikawa *et al.* 2002)

Anti-inflammatory effects on the peripheral blood of subjects with inflammatory bowel disease (IBD) who consumed probiotic yogurt were assessed by Lorea *et al.* 2007. We studied 20 healthy controls and 20 subjects with IBD, 15 of whom had Crohn’s disease and five with ulcerative colitis were studied. All the subjects consumed *Lactobacillus rhamnosus GR-1* and *L. reuteri RC-14* supplemented yogurt for 30 days. Probiotic yogurt intake was associated with significant anti-inflammatory effects that paralleled the expansion of peripheral pool of putative T(reg) cells in IBD patients and with few effects in controls.

The benefit of LAB on crohn’s disease was also attributed to the stimulation of the IgA response. Malin *et al.* 1996 suggest that oral bacteriotherapy using *L.caseii* can restore antigen-specific IgA immune response in persons with crohn’s disease.

### 1.2.5. EFFECT ON HELICOBACTER PYLORI

Yoghurt-derived food preparations could become simple and inexpensive therapies to suppress *H. pylori* infections in endemic countries (Oh *et al.*2002). Evidence suggests that ingesting lactic acid bacteria exerts a suppressive effect on *Helicobacter pylori* infection in both animals and humans. Supplementing with *Lactobacillus*- and *Bifidobacterium*-containing yogurt was shown to improve the rates of eradication of *H. pylori* in humans (Wang *et al.* 2004).

Several in vitro and animal studies have shown reduced viability of *H.pylori* and less adhesion of the bacteria to human intestinal mucosal cells after treatment with various *Lactobacillus* strains (Aiba *et al.* 1998).

Midolo *et al.* 1995 showed that the growth of *H.pylori* was inhibited by lactic acid in a pH-independent manner. Armuzzi *et al* 2001 reported that when 120 asymptomatic subjects who were positive for *H.pylori* infection received an *L.caseii* strain GG supplement over 14

days period in addition to a standard 1 week antibiotic therapy regimen, the eradication of *H.pylori* was faster than that in control subjects.

### 1.2.6. EFFECT ON IMMUNOMODULATORY ON CELL MEDIATED IMMUNITY

Vesely *et al.* 1985; perdigon *et al.* 1987 reported increased lymphocytic activity in mice fed with fermented milks containing 50 g/d of viable cultures of *S.thermophilus* and *L.acidophilus* for 8 days.

In a comprehensive in vivo experiment by Desimone *et al.* (1989 a) involving 17 human subjects of 21-36, who were challenged with yoghurt bacteria, a progressive increase in the serum gamma –interferon was observed. The average gamma –Interferon level was 0.42 U.I/ml initially, which increased to 0.70 U.I/ml after 28 days of consumption of *L.bulgaricus* and *S.thermophilus* at the dose of  $3 \times 10^{12}$  cell/day.

An in vitro experiment conducted earlier by the same workers, using human peripheral blood lymphocytes, showed that the addition of small quantity of yoghurt containing live cells, increased the production of gamma –interferon by 3-4 times (Desimone *et al.* 1989b). The observation is highly significant, because the heated yoghurt did not show any stimulation. However, concanavalin –A, a T-cell mitogen and yoghurt synergistically further increased the gamma –interferon levels as compared to individuals. Along with an increase in the quantity of gamma-interferon, a corresponding increase in the number of the killed K 562 tumor cells by the peripheral blood lymphocytes also increased. The heat-treated yoghurt had clearly inferior immunopotentiating properties, compared to the natural yoghurt, which indicated the importance of live lactic acid bacteria.

Lactobacillus supplementation significantly improved the immune status and growth status of pre-school children. (Sucharitha devi S and Yasoda devi P 1998)

### EFFECT ON HUMORAL IMMUNITY

A study by Perdigon *et al.* 1995, found that yoghurt stored upto 20 days could increase the anti sheep red blood cells antibodies in mice, but in mice compared to fresh yoghurt feeding, the specific IgA against *S. typhimurium* increased only with fresh yoghurt. With respect to total immunoglobins, the stored yoghurt had no effect.

Conge *et al.*, 1980 were the first to observe an increase in the level of immunoglobins, belonging to Ig G2a class, in serum of mice fed a diet supplemented with yoghurt, containing live lactic acid bacteria. Similar results were reported by other workers (Vesely 1985; De simone *et al.* 1989a).

While monitoring humoral immunity of yoghurt fed mice, Vesely *et al.* 1985 observed that Ig G2a and IgM increased transiently on the 15<sup>th</sup> day of feeding yoghurt and heated yoghurt as compared to control group. However, on the 30<sup>th</sup> day, all the groups were at par for all types of

immunoglobins. Significantly higher antibody level was observed in germ-free mice, when fed yoghurt containing live bacteria, as compared to those fed heated yoghurt for a period of 8 days (Wade *et al.* 1984).

In another study, when different species of lactic acid bacteria were used for feeding. Only *Lb. delbrueckii subsp. Bulgaricus* showed higher level of antibody titre (Moineau and Goulet 1991).

### 1.2.7. EFFECT ON VITAMIN CONTENT AND CANDIDAL INFECTIONS

Laxminarayan, 1976 Studied the vitamin content of yoghurt prepared with different cultures revealed differences between species and between single and mixed cultures. Thiamine and riboflavin contents were decreased when single cultures were used. Folic acid activity of yoghurt was higher than in milk while vitamin B<sub>12</sub> content was reduced in all cases. Use of mixed cultures generally resulted in increasing the riboflavin and folic acid contents.

Elmadfa *et al.* (2001) observed that the viable bacteria of probiotic yoghurt influence the parameters of the B vitamins (B1, B2 and B6) status in healthy adult humans. Machrzak and Elmaifa, 2002 reported increase in content of vitamin Thiamin, riboflavin and B6 of eight different sorts of commercially available probiotics yoghurt prepared with starter cultures that are normally used and additionally enriched with *Lactobacillus casei*, *Lactobacillus acidophilus*, *bifidobacteria*.

A threefold decrease in infections was seen when patients consumed yogurt containing *Lactobacillus acidophilus*. Daily ingestion of 8 ounces of yogurt containing *Lactobacillus acidophilus* decreased both candidal colonization and infection (Hilton *et al.* 1992).

### CONCLUSION

Yoghurt is considered by nutritionists to be a very nutritious and healthy food compared to milk. Probiotic cultures are live bacteria which help in better absorption of nutrients. They play an important role in reduction of serum cholesterol, alleviation of lactose intolerance, reduction of diarrhea, prevention and suppression of colon cancer, stimulation of the immune system etc. Yoghurt is prepared by fermenting milk with starter cultures containing different types of probiotics, normally *streptococcus thermophilus* and *lactobacillus bulgaricus*. Use of different probiotic blends in combination has several health benefits. Yoghurt can also be prepared with incorporation of fruits pulp /essence to improve the taste and flavor and to have an additional benefit to human nutrition.

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