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2.1.2 Legal requirement for yoghurt

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2.5 Sodium alginate
2.1 Natural (Plain) Yoghurt:

2.1.1 Definition:

F.A.O/W.H.O (1977), stated “Yoghurt is coagulated milk product obtained by lactic acid fermentation through the action of *Lactobacillus Bulgaricus* and *Streptococcus Thermophilus*, form milk and milk products (pasteurized or concentrated milk) with or without optional additions (milk powder, skim milk powder, whey powder etc.). the microorganisms in the final products must be viable and abundant”.

Mckinley (2005) defined, “Yoghurt is one of the most popular fermented milk products worldwide and has gained widespread consumer acceptance as a healthy food. It provides an array of nutrients in significant amounts, in relation to its energy and fat content, making it a nutrient-dense food. In particular, yoghurt can provide the body with significant amounts of calcium in a bioavailable form. Furthermore, yoghurt has many health benefits beyond the basic nutrition it provides, such as improved lactose tolerance, a possible role in body weight and fat loss, and a variety of health attributes associated with probiotic bacteria’.

2.1.2 Legal requirement for yoghurt

As per Prevention of Food Adulteration act, 1954 (CII, 2006), yoghurt means a coagulated milk by lactic acid fermentation through *lactobacillus delbruckii var. Bulgaricus* & *Streptococcus thermophilus*. It may also contain cultures & if added the declaration to this effect shall be made on the label. The microorganism in the final product must be viable and abundant. The product shall have smooth body and custard like consistency with no whey separation. It may also contain (i) milk powder, skinned milk powder, unfermented buttermilk, concentrated whey, whey powder, whey protein, whey protein concentrates, water soluble milk proteins, edible casein and caseinates manufactured from pasteurized products and lactose enzyme preparation; (ii) Sugar, corn syrup or glucose syrup in case of sweetened flavoured and fruit yoghurt only: (iii) Fruit, fruit pulp, jam, fruit syrup, fruit juice etc; (iv) permitted colours and flavoures in flavoured and fruit yoghurt only.

It may contain permitted stabilizer up to a maximum limit of 0.5 percent, by weight. It shall also conform to the following standard, namely,
2.1.2 A/c to PFA Standard, Legal requirement for yoghurt:

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Yoghurt Plain</th>
<th>Yoghurt Skimmed</th>
<th>Yoghurt Sweetened/Flavoured</th>
<th>Fruit Yoghurt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total milk solids % by weight not less than</td>
<td>13.5</td>
<td>11.0</td>
<td>13.5</td>
<td>10.0</td>
</tr>
<tr>
<td>Milk fat % by weight not less than</td>
<td>3.0</td>
<td>0.5</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Sugar % by weight not less than</td>
<td>_</td>
<td>_</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Protein % by weight not less than</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Titratable acidity (%)</td>
<td>Titratable acidity of the product shall be from 0.8 to 1.2% by weight (as lactic acid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial count (per gram)</td>
<td>Bacterial count shall not be less than 10,00,000.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coliform count (per gram)</td>
<td>Escherichia coli shall be absent in the product</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Titratable acidity of the product shall be 0.8 to 1.2 percent by weight (as lactic acid). The specific lactic acid bacteria count per gram of the product shall not be less than 10,00,000 and *Escherichia coli* shall be absent in the product. The type of yoghurt shall be clearly indicated on the label, otherwise standard of plain yoghurt shall apply.

2.1.3 Health Benefits of Yoghurt:

*Ringdahl* (2001) showed that regular ‘Super Yogurt’ consumption relieves by 3 fold Vaginal Yeast Infection that affects every women at least once in their lives. Practitioners claim that applying plain/natural ‘Super Yogurt’ in the vagina will cure Candidasis as the Beneficial Bacteria feed on the yeast organisms. If one is pregnant, the yeast infection will be passed on to the baby who will get it in its throat and intestinal tracts called Thrush. Also, the irritation/Yeast Infection will allow virus easier entrant into one’s blood stream thus making one more susceptible to contracting HIV (AIDS) or other Sexually Transmitted Diseases if left untreated.

*Heaney et al.* (2002) concluded that for young girls going through the rapid growth spurts of puberty, getting calcium from dairy products, such as yogurt, might be better for building
bone than taking a calcium supplement. Finnish researcher’s enrolled 195 healthy girls aged 10-12 years and divided them into 4 groups. One group was given supplemental calcium (1000 mg) + vitamin D3 (200 IU) each day. The second group received only supplemental calcium (1000 mg/day). The third group ate cheese supplying 1,000 mg of calcium each day, and the fourth group was given a placebo supplement.

At the beginning and end of the study, DEXA (dual-energy X-ray absorptiometry) scans were run to check bone indexes of the hip, spine, and whole body, and the radius and tibia were checked by peripheral quantitative computed tomography.

At the conclusion of the study, girls getting their calcium from cheese had higher whole-body bone mineral density and cortical thickness of the tibia than girls given supplemental calcium + vitamin D, supplemental calcium alone, or placebo. While the researchers noted that differences in the rate at which different children naturally grow might account for some of the differences seen in bone mineral density, they concluded: "Increasing calcium intake by consuming cheese appears to be more beneficial for cortical bone mass accrual than the consumption of tablets containing a similar amount of calcium."

Canzi et al, (2002) studied on the effect of yoghurt as a dietary supplement which was investigated with regard to the gut ecosystem and lipid metabolism of 12 healthy, elderly people (78.3 ± 9.8 years, body mass index 23.6 ± 5.3 kg.m⁻², mean ± SD). Commercial yoghurt with homogenized fruit was prepared by fermenting milk with yoghurt specific cultures Lactobacillus delbrueckii ssp. bulgaricus (strain AY/CSL) and Streptococcus thermophilus (strain 9Y/CSL). The subjects consumed their usual diet (equal to 62796698 kJ.d⁻¹) over a 2-week baseline period (baseline start to end) and then were supplemented for 4 weeks with 250g.d⁻¹ of fruit yoghurt. The yoghurt was administered in125 g portions twice per day: at breakfast in substitution of milk and in the afternoon in substitution of tea with milk (test). At the end of the 4-week period the volunteers returned to their usual diet for a further 4 weeks (follow-up). At the end of each trail period no changes were observed in faecal water content, pH, bile acid concentration or cytolytic activity of the faecal water. Throughout the study there was significant variation neither in dietary intake of macro and micronutrients, nor in the plasma lipids and during the experimental period, in the counts of the total anaerobic microorganisms, bifidobacteria, lactobacilli, coliforms or enterococci. The only significant difference was observed in the clostridia counts that decreased (P < 0.05) after the consumption of yoghurt. Moreover, this effect was still evident at the end of the
colon ecosystem, as clostridia are involved in the production of putrefactive compounds, it is possible that a yoghurt-supplemented diet can maintain and/or improve the intestinal microbiota of elderly subjects.

Boeneke (2003) showed that folic acid fortification is used in the prevention of neural tube defects such as spina bifida and anencephaly, heart defects, facial clefts, urinary tract abnormalities, and limb deficiencies. Although yogurt is not a good source of folic acid, fortification could aid in prevention of above mentioned defects. Fortification of yogurt with folic acid may or may not change its physico-chemical characteristics. Fat free sugar free yogurt was manufactured using 0, 25%, 50%, 75% and 100% of the recommended daily allowance of 400 micrograms of folic acid. Treatments included addition of folic acid at these levels before and after pasteurization. Lemon and strawberry flavourings were added to improve flavour and improve colour of yogurts. The objective was to examine the effects of folic acid on viscosity, pH, TA, syneresis, colour, composition, and folic acid concentration in the product at one, three, and five week intervals. Data were analyzed using the General Linear Model procedure with a general linear model with repeated measures in time analysis by the Statistical Analysis System. Significant differences were determined at $P<0.05$ using Tukey’s Studentized Range Test. There were no differences in the electrophoretic mobilities of the protein/peptides in the samples. Mean flavour scores were higher for lemon and strawberry yogurts as compared to plain when tested by a trained sensory panel. Folic acid fortification of yogurt impacted some of its physico-chemical attributes.

Adolfsson et al, (2004) reported that in recent years, numerous studies have been published on the health effects of yoghurt and the bacterial cultures used in the production of yoghurt. In the United States, these lactic acid-producing bacteria (LAB) include Lactobacillus and Streptococcus species. The benefits of yoghurt and LAB on gastrointestinal health have been investigated in animal models and, occasionally, in human subjects. Some studies using yoghurt, individual LAB species, or both showed promising health benefits for certain gastrointestinal conditions, including lactose intolerance, constipation, diarrheal disease, colon cancer, inflammatory bowel disease, Helicobacter pylori infection and allergies. Patients with any of these conditions could possibly benefit from the consumption of yoghurt. The benefits of yoghurt consumption to gastrointestinal function are most likely due to effects medicated through the gut microflora, bowel transit and enhancement of gastrointestinal innate and adaptive immune responses. Although substantial evidence
currently exists to support a beneficial effect of yoghurt consumption on gastrointestinal health, there is inconsistency in reported results, which may be due to differences in the strains of LAB used, in routes of administration, or in investigation procedures or to the lack of objective definition of “gut health”. Further well-designed, controlled human studies of adequate duration are needed to confirm or extend these finding.

Zemel et al. (2005) indicated that adding one or two serving of yogurt to your daily diet can help you maximize loss of fat and minimize loss of muscle the optimal outcome for any diet.

Fabian and Elmadfa (2006) found that one group of 17 women consumed 3 ounces (100 g) a day of probiotic yogurt, while a second group of 16 women were given 3 ounces of conventional yogurt daily for 2 weeks. Then both groups were given 6 ounces (200 g) of the type of yogurt they had been consuming for 2 more weeks. The study ended with a final 2 weeks during which both groups of women ate no yogurt. In the women consuming probiotic yogurt, not only did levels of LDL (bad) cholesterol decrease significantly, but their HDL (good) cholesterol substantially increased. Women consuming conventional yogurt also experienced a significant drop in LDL cholesterol, although their HDL did not rise. The take-home message: adding a daily cup of yogurt-preferably a yogurt with probiotic bacteria-to your healthy way of eating is an easy and delicious way to improve your cholesterol profile.

Tudor and Havranek (2009) showed that fermented milk is the most popular group of functional food. This paper looks at the nutritional value and health benefits of fermented milk which include the improvement of lactose metabolism, the prevention of cardiovascular disease, osteoporosis and tumours and maintaining the optimum body weight. Nutritive value of fermented milk mostly depends on nutritive value of milk as a raw material as well as on used microbial culture. However, their nutritive value and thereby their health benefits can be increased by adding probiotic microorganisms, milk and whey powder, fruit additives, fibres or vitamins

2.1.4 A/c to PFA Standard, yoghurt composition derived as:

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Name of the product</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plain Yoghurt</td>
<td>Yoghurt Skimmed</td>
<td>Yoghurt Sweetened flavour</td>
<td>Fruit Yoghurt</td>
</tr>
<tr>
<td>Total milk solids</td>
<td>NLT 13.5</td>
<td>11.0</td>
<td>13.5</td>
<td>10.0</td>
</tr>
<tr>
<td>(% weight)</td>
<td>NLT 3.0</td>
<td>NMT 0.5</td>
<td>NLT 3.0</td>
<td>NLT 1.5</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Milk fat (% by weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar (% weight)</td>
<td></td>
<td></td>
<td>NLT 6.0</td>
<td>NLT 6.0</td>
</tr>
<tr>
<td>Titratable acidity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial count (per gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coliform count (per gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Titratable acidity of the product shall be from 0.8 to 1.2 % by weight (as LA)

Bacterial count shall not be less than 10,00,000.0

Escherichia coli shall be absent in the product

(Source: The Prevention of Food Adulteration Act & Rules 2004.)

2.1.5 PFA standards of microbiological analysis of yoghurt and dahi.

<table>
<thead>
<tr>
<th>Requirements</th>
<th>Yoghurt/Dahi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific lactic</td>
<td>Bacterial count not less than 10,00,000/g</td>
</tr>
<tr>
<td>Total plate count</td>
<td>Not more than 10,00,000/g</td>
</tr>
<tr>
<td>Coliform test</td>
<td>Not more than 10/g</td>
</tr>
<tr>
<td>E.coli</td>
<td>Absent in 1g</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Absent in 25g</td>
</tr>
<tr>
<td>shigella</td>
<td>Absent in 25g</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Not more than 100/g</td>
</tr>
<tr>
<td>Yeast and mold count</td>
<td>Not more than 100/g</td>
</tr>
<tr>
<td>Anaerobic spore count</td>
<td>Absent in 1g</td>
</tr>
<tr>
<td>Listeria monocytogens</td>
<td>Absent in 1g</td>
</tr>
</tbody>
</table>

(Source: The prevention of Food Adulteration Act & Rules 2004)
2.1.6 A/c to CODEX Standard:

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Yoghurt, Alternate Culture Yoghurt and Acidophilus milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk protein (a) (%m/m)</td>
<td>Min. 2.7%</td>
</tr>
<tr>
<td>Milk fat (%m/m)</td>
<td>Less than 15%</td>
</tr>
<tr>
<td>Titratable acidity, expressed as %lactic acid (%m/m)</td>
<td>min. 0.6%</td>
</tr>
<tr>
<td>Sum of microorganisms constituting the starter culture defined in section 2.1 (cfu/g, in total)</td>
<td>min. $10^7$</td>
</tr>
<tr>
<td>Labelled microorganisms (b) (cfu/g, total)</td>
<td>min. $10^8$</td>
</tr>
<tr>
<td>Yeasts (cfu/g)</td>
<td>-</td>
</tr>
</tbody>
</table>

(Source: Codex Standards for fermented milks codex STAN 243-2003)

2.1.7 Different compositions for manufacturing yoghurt:

Kadamany et al. (2002) reported that strained yogurt, labneh, produced by straining cow’s milk set yogurt in cloth bags, was stored at 5, 15, and 25°C, and changes in microbial counts, pH, titratable acidity, percentage of free whey, and sensory attributes were monitored during storage. Counts of total aerobes, psychrotrophic yeasts, yeasts and moulds, and lactic acid bacteria, except in samples stored at 25°C, increased irrespective of storage temperature. The pH of samples decreased, titratable acidity and percentage of free whey increased, and texture defects were detected at a later stage than flavour changes during storage. Shelf-life data of labneh was adequately described by the Weibull distribution. The nominal shelf life determined using sensory changes and yeast counts as failure criteria ranged from 8.5 to 10.5, 4.7 to 5.8 and 2.3 to 2.7 d at 5, 15 and 25°C, respectively. Q10 (shelf life at T°C/shelf life at T+10°C) for flavour quality loss was 1.98 at 5°C, and the corresponding activation energy was 11.3 kcal/mol.

Massey (2002) took thirty-two healthy, normolipemic male college students eating in a single dining hall who participated in a study designed to ascertain the effect of changing milk consumption on nutrient intake and lipoprotein. The men drank no milk for 3 wk, then 1500 ml milk with 2% fat daily for 3 wk, their usual diet for 2 wk, no milk again for 3 wk, 1250 ml non fat milk daily for 3 final wk. Similarly, 30 female college students consumed either 480
ml low fat yogurt, then no yogurt for 4 wk each in a crossover design. Body weight and physical activity were not different among dietary treatments. Protein, energy, and calcium intake varied significantly with changes of milk consumption. Total dietary fat decreased significantly when no milk or non-fat milk was consumed, whereas dietary cholesterol was significantly lower only when non-fat milk was consumed. Yogurt supplementation significantly increased intake of calcium and carbohydrate. Although some serum lipid means differed significantly among some sampling points, there was no effect on total cholesterol, total triglycerides, high density lipoprotein cholesterol, or distribution of electrophoretic lipoprotein fractions that could be attributed to changing milk or yogurt consumption.

Ryan et al. (2004) stated six popular fruit flavours were formulated into the yogurt beverages. Difference and preference tests were conducted to select the most popular flavour type, flavour intensity, and sweetener type and percent. The two top flavours were presented to a minimum of 30 consumers in each of five age groups. Results indicated that the beverages were "liked" on a line-hedonic scale with a range of 1 to 24 (24 for like extremely).

Alm (2004) studied in vitro digestibility of fermented milk products were studied. Size of curds at various stages of digestion was determined by a screening method, and the amount of nitrogen in each curd size was estimated by analyzing lyophilized curd samples. Results were compared with human milk which, having the best digestibility properties was reference. Evaluation showed that low pH, especially as a result of fermentation, had a positive influence on in vitro digestibility. Yogurt and also acidophilus and bifidus milk, in spite of much higher protein content, resembled human milk fairly well in digestibility. Fermented milk products could be provided as easily digestible foods and tolerated by infants, children, and adults even when gastrointestinal disturbances prevail.

Rashmikant and Raymond (2007) reported that yoghurt, a healthful dairy product, is popular throughout the world and benefits of dietary fibers are known since vedic times. Perceiving the potential of yoghurt and dietary fiber combination as a high value product project was conceived with the aim of developing fiber fortified yoghurt as a ‘Functional food’. The fiber fortified yoghurt, so obtained, had 24.8% TS, 3.10% fat, 2.98% protein, 2.29% lactose, 7.21% sucrose and 1.11% total ash. The product packaged in polystyrene cups, had a shelf life of more than two weeks at 5±1°C. Yoghurt containing the selected fibers was spray dried to a powdered product. Streptococcus thermophilus showed 41.66%
and 62.25% viability after spray drying of control, yoghurt and fiber fortified yoghurt respectively. Acetaldehyde retention was 46.42% in control yoghurt and 81.07% in fiber fortified yoghurt. The fiber fortified yoghurt powder showed 92.92% dispersibility, 5.8 ml insolubility index, 12.89 wetting time, 0.46 g/ml loose bulk density, 0.76 g/cc packed density and 56.15 angle of response. The yoghurt powder had 5.09% moisture, 14.2% fat, 15.88% protein, 9.38% lactose, 32.54% sucrose and 5.10% total ash. The acceptability of the nearly similar for both the control (6.5) and fiber fortified yoghurt (6.3).

**Guggisberg et al. (2008)** investigated the effect of inulin addition (0-4%) upon texture and microstructure of set yoghurt with different levels of fat (0.2%-3.5%). A two-factor experimental design with four treatments was used for data analysis. Skimmed milk with various inulin and cream concentrations was standardized to 4% protein content, homogenized, heated to 92°C and fermented at 42°C until a pH of 4.6 was reached. The chemical composition pH, consistency and microstructure properties of the yoghurts were analysed after 6 days of storage at 5°C. The statistical analysis showed that inulin and fat significantly affected the rheological and sensory results. Higher yield stress, “firmness” and “creaminess” values were observed in yoghurt produced with higher inulin additions, whereas the pH value was not affected. A significant correlation was found between yield stress and sensory determined firmness(r=0.91). The microstructure examined by confocal laser scanning microscopy (CLSM) was only slightly affected by the concentrations of inulin in the range studied, possible due to weak protein interactions between the inulin and the milk protein network.

**Vaghela et al. (2008)** showed that proper body and texture minimum wheying off and characteristics acidic flavour are the essentials of set yoghurt of good quality. These characteristics are influenced by the composition especially solid level, of the milk used for yoghurt production. Hydrocolloid stabilizers are permitted additives. This, through their hydrophilic properties, may improve the body and texture and consistency of yoghurt. The beneficial effect of pectin and alginate on quality of set skim milk yoghurt through improvement in its rheological properties was offset by their negative impact on flavour of the product; diacetyl was less in skim milk yoghurt compared to the product with 3% fat. Use of stabilizers resulted in further reduction in diacetyl production. In yoghurt with 3% fat, improvement in overall sensory score was noticed both with pectin and alginate probably
because in this case the negative effect of stabilizer on flavour production was contributed to production and perception of flavour.

Renan et al. (2008) studied that the rheological properties of stirred yoghurt as a function of the delay between milk heat-treatment and inoculation (0, 1 and 2 days), of pH in the acid gel on stirring (4.4, 4.7 and 5.0), of the storage temperature (4, 12 and 20ºC) for 24 h following stirring and of over-acidification (allowed or inhibited). At low pH values, the gels exhibited the higher elastic modulus (G') and fracture strength. They yielded stirred yoghurts with higher G' and viscosity, and higher increase in G' and viscosity during storage (“rebodying”). Rebodying was only partially explained by over-acidification and cooling. Changing the storage temperature had no impact on the evolution of G' after stirring; Hydrophobic interactions were therefore probably not involved in rebodying. Electrostatic interactions seemed to play a major role in rebodying, as pH on stirring was the significant factor.

Vahedi et al. (2008) found that the effect of "Osmodehydrofrozen" fruits on sensory, physical, chemical and microbiological properties of yoghurt and its quality during storage was evaluated. This research was done in two stages. At the first stage, the fruit percentage, type and addition time (before and after of fermentation) was determined, the results indicated that yoghurts which contained 10% apple or 13% strawberry and added after fermentation had better quality. Because of high osmotic activity of apple, the synersis value was lower in apple yoghurt. According to osmotic activity in both fruits, the synersis value was very lower than fruit yoghurts which contained untreated fruits. Taste value was higher in strawberry yoghurt and texture and mouth feel values was higher in the yoghurt with high percentages of fruit. The results of second stage (quality evaluation during storage) indicated that storage had significant effect on pH, acidity, synersis, taste and texture (P<0.05). The sample contained apple didn't have any mould and yeast; Coliforms disappeared after 7 days of storage. In samples which contained strawberry, yeasts were grown and coliforms disappeared after 7 days of storage.

Hassan and Amjad (2009) studied that yoghurt was prepared with two different types of starter cultures that are Lactobacillus bulgaricus and L. acidophilus. In this study 3, 4 and 5% starter cultures were used and stored at 4ºC for 12 days. To analyze the effect of two different cultures and their concentrations on the properties of yoghurt, different physio-chemical tests (protein, lactose, ash, fat, acidity, total solid, pH and moisture) were performed. The results
showed that the protein, lactose, ash, fat, acidity and total solid mass were slightly increased while pH and moisture values gradually decreased during storage period of 12 days. The comparative study of starter cultures showed that L. acidophilus produced good quality yoghurt as compared to L. bulgaricus.

**Pinto et al. (2009)** evaluated the production of acetaldehyde, diacetyl and ethanol in whole plain yoghurts manufactured with commercial starter cultures, yoghurt acquired in a local market, and milk fermented by a single culture of either *Streptococcus thermophilus* or *Lactobacillus delbrueckii ssp. bulgaricus*. The headspace technique was used for sample preparation, following identification and quantification by gas chromatography. During an 8-h incubation period, mixed cultures were the most efficient in lowering the pH (from 6.30 to 4.8), followed by *S. Thermophilus* (from 6.30 to 5.18) and *L. bulgaricus* (from 6.30 to 5.8). During the storage period, however, a single culture of decreased the pH more than *S. Thermophilus*, but still less than the mixed commercial cultures. Plain yoghurts acquired in the market, those made with commercial starter cultures, and fermented milks obtained with single cultures showed, after 21 days of storage, concentrations of acetaldehyde from 11 to 35 mg/L, and of diacetyl from 0 to 0.85 mg/L. An increasing concentration of ethanol was observed during the storage period, and its production was observed even in the incubation stage of all products. It was also observed that the acetaldehyde concentration was inversely correlated to ethanol production in some products. The combination of headspace, identification and quantification techniques by gas chromatography in this work was efficient in the identification and quantification of the major aromatic compounds and ethanol content of yoghurt.

**Kanake et al. (2009)** found out the optimum level of skim milk powder that could be incorporated to obtain the best quality yoghurt with acceptable flavour, body and texture. Addition of skim milk powder at 4% and 0.3% gelatin in the fresh milk after pre-heating. The yoghurt prepared with 4% SMP (Skim milk powder) was sensorily superior to that of control sample. The final product contained 4.18% fat, 5.41% protein, 26.39% total solids, 0.90% acidity and 73.61% moisture.

**Oyeleke (2009)** found that five samples each of twenty brands of commercially produced yoghurt were purchased randomly from different provision stores within Minna. The results showed that the total bacterial count ranged from $1.0 \times 10^7$ to $9.4 \times 10^7$ cfu/ml. The organisms isolated included species of *Staphylococcus, Lactobacillus, Enterobacter* and
Bacillus, for bacteria, and species of Aspergillus, Fusarium, Candida, Penicillium, Cephalosporium and Mucor for fungi. However, species of Bacillus and Aspergillus were isolated the most frequently. The result revealed that yoghurt commercially produce in Minna are of high quality. All effort should be geared toward sustaining it.

Rhaman and Shuai (2009) determined the microbiological and biochemical changes that occur during fermentation of camel milk inoculated with each of five selected starter cultures at 43°C for 6 h, as well as the sensory evaluation of the products. The total viable counts of the starter cultures throughout fermentation period (6 h) showed that the combination of Lactobacillus bulgaricus CH2 plus Streptococcus thermophilus 37 (1:1) had more counts and produce more acid (lower pH) compared to the single starter cultures. Also when comparing the different treatments, the amount of FAG released after 6 h was highest in the mixed starter cultures than in the corresponding single starter cultures. The final fermented milk products were free from pathogenic bacteria such as Salmonella spp, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli O157:H7 and Bacillus cereus, while the total coliforms, yeasts and moulds counts were less than 10 cfu per ml. The results of the sensory evaluation study indicated that the camel milk fermented by mixed yogurt culture was the most accepted while the one fermented by Lactococcus lactis was the least. However, the consistency of all fermented camel milk products was watery and showed a fragile, poor structure (poor scores). In general mixed yogurt culture showed superior growth, acid production and proteolytic activity than single starter cultures and acceptable fermented camel milk.

Ciron et al. (2009) compared the effect of high-pressure homogenization uses a microfluidizer on texture, water holding capacity, and extent of syneresis on stirred yoghurts with that of conventional homogenization. The effect of homogenization condition on particle size was also assessed in milk and in yoghurt. Stirred yoghurts were prepared from recombined milk samples (0 and 1.5% fat) heat-treated (9.5 °C, 2 min) and then treated by conventional valve homogenization at 25 MPa or micro fluidization at 150 MPa. Homogenization conditions influenced the particle size in milk, gel particle size, and textural quality of stirred yoghurts in a manner dependent upon fat content. Milk micro fluidized at 150 MPa had small particle size than homogenized milk, but resulted in larger particles in yoghurt. Micro fluidization of low-fat milk modified the microstructure of yoghurt, giving more interconnectivity in the protein networks with embedded fat globules, but with similar
texture profiles and water retention compared with yoghurt made from conventionally homogenized milk.

Atasoy (2009) showed that plain and fruit-flavoured yoghurts were made by adding 2.5, 5.0, 7.5, 10.0 ml carob juice concentrate (CJC) proteins to 100 ml milk. The titratable acidity, pH, viscosity, whey separation, yoghurt organisms and sensory properties were determined weekly over a period of 4 weeks. Addition of CJC caused an increase in the fermentation time and reduced viscosity and viable organisms. While increase the pH and whey separation of the yoghurts (P<0.05). A lack of sweetness was the main criticism of the yoghurts with 2.5 and 5.0 ml CJC, while those with 7.5 or 10.0 ml were mostly preferred by sensory panellists.

Ashmawy and Ibrahim (2009) showed that yeast and moulds are little affected by low pH and may cause spoilage of yoghurt during storage. In the present study, potassium sorbate was added as a preservative in concentrations of 0.05%, 0.1 % and 0.2%. The unpreserved yoghurt showed unfavourable characteristics: yeast and mould counts and acidity increased. The addition of potassium sorbate, however, inhibited yeast and mould levels, with normal characteristic properties extending more than 14 days. Potassium sorbate in thus seen as valuable for preserving yoghurts.

Horiuchi et al. (2009) found that yoghurt starters Lactobacillus delbrueckii Ssp. bulgaricus and Streptococcus thermophilus are well known facultatively anaerobic bacteria that can grow in oxygenated environments. We found that they removed dissolved oxygen (DO) in a yoghurt mix as the fermentation progressed and that they began to produce acid actively after the DO concentration in the yoghurt mix was reduced to 0 mg/kg, suggesting that the DO retarded the production of acid. Yoghurt fermentation was carried out at 43 or 37°C both after the DO reduction treatment and without prior treatment. Nitrogen gas was mixed and dispersed into the yoghurt mix after inoculation with yoghurt starter culture to reduce the DO concentration in the yoghurt mix. The treatment that reduced DO concentration in the yoghurt mix to approximately 0 mg/kg beforehand caused the starter culture LB81 used in this study to enter into the exponential growth phase earlier. Furthermore, the combination of reduced DO concentration in the yoghurt mix beforehand and incubation at a lower temperature (37°C) resulted in superior set yoghurt with a smooth texture and strong curd structure.

Dobrea et al. (2009) investigated that yoghurt samples revealed a decrease with approximately 1 log in bacterial count for Streptococcus thermophilus, during the shelf life of
this product. The bacterial count was maintained above the minimal level of 106 UFC/ml, which is considered the minimal probiotic bacteria concentration that could still have benefits for consumer's organism.

Peng et al. (2009) stated that casein interaction plays an important role in the textural properties of yoghurt. The objective of this study was to investigate how the concentration of insoluble calcium phosphate (CCP) that is associated with casein particles and the length of fermentation time influence properties of yoghurt gels. A central composite experimental design was used. The initial milk pH was varied by preacidification with glucono-δ-lactone (GDL), and fermentation time (time to reach pH 4.6 from the initial pH) was altered by varying the inoculum level. We hypothesized that by varying the initial milk pH value, the amount of CCP would be modified and that by varying the length of the fermentation time we could influence the rate and extent of solubilization of CCP during any subsequent gelation process. We believe that both of these factors could influence casein interactions and thereby alter gel properties. Milks were preacidified to pH values from 6.55 to 5.65 at 40°C using GDL and equilibrated for 4 hr before inoculation. Fermentation time was varied from 250 to 500 min by adding various amounts of culture at 40°C. Gelation properties were monitored using dynamic oscillatory rheology, and microstructure was studied using fluorescence microscopy. Whey separation and permeability were analyzed at pH 4.6. The preacidification pH value significantly affected the solubilization of CCP. Storage modulus values at pH 4.6 were positively influenced by the preacidification pH value and negatively affected by fermentation time. The value for the loss tangent maximum during gelation was positively affected by the preacidification pH value. Fermentation time positively affected whey separation and significantly influenced the rate of CCP dissolution during fermentation, as CCP dissolution was a slow process. Longer fermentation times resulted in greater loss of CCP at the pH of gelation. At the end of fermentation (pH~4.6), virtually all CCP was dissolved. Preacidification of milk increased the solubilization of CCP, increased the early loss of CCP cresslinks, and produced weak gels. Long fermentation times allowed more time for solubilization of CCP during the critical gelation stage of the process and increased the possibility of greater casein rearrangements; both could have contributed to the increase in whey separation.

Yuksel and Erdem (2010) investigated the effect of transglutaminase (Tgase) on the functional properties of set yoghurt, due to enzymatic cross-linking of milk proteins. Yoghurt
samples were prepared from three formulations: reconstituted skim milk, and whole milk containing two different solid non-fat (SNF) content, with four enzyme treatments: control (no enzyme) and three microbial Tgase treatments: pre-incubated 90 minutes, pre-incubated and inactivated after 90 minutes not pre-incubated. The modifications which were caused by Tgase were found to be more clear when Tgase was active in set type yoghurts. It was found that non-fat yoghurt sample with lower SNF content can be produced with improved textural properties using Tgase.

Khan and Abraham (2010) reported that some properties, namely; viscosity, flavour, acidity, texture, aroma and palatability of cultural yoghurt made from milk previously heated to 90°C for 30 minutes in vat were studied and the results compared to those of yoghurt fortified by addition of dry skim milk powder. The results showed no significance difference (P<0.05) between the two types of yoghurt regarding viscosity, and syneresis during the first 4 days, after which the yoghurt fortified with skim milk powder, tended to whey off, becoming grainy in texture, declining in viscosity and developing high acidity (2.0 to 2.5 % LA). A t-test analysis (p<0.05) indicated that the two kinds of yoghurt were similar on average. Panellist however, found the fortified yoghurt harsher and inferior in flavor. Sensory evaluation indicated that on average, the unfortified sample of yoghurt made from milk preheated to 90°C for 30 minutes was superior. Besides, the cost of adding extra solids to the fortified yoghurt made it 4.5 % more expensive which was discouraging to the ordinary consumers.

Matumoto - Pintro et al. (2010) substituted the native and denatured whey protein ingredients for milk protein in yoghurt formulations which resulted in excessive elasticity and grainy texture. These characteristics were incompatible with the smooth and short texture expected from yoghurt. Whey protein ingredients were modified to produce yoghurts with acceptable texture properties. Alteration of the ratio of α-lactalbumin to β-lactoglobulin, heat denaturation and hydrolysis treatments were applied to whey protein to improve their behaviour in yoghurt formulations. Ingredients with increased proportions of α-lactalbumin or made from partially hydrolyzed protein produced yoghurts that closely matched the characteristics of control yoghurt. The effect of whey protein ingredients on yoghurt rheological properties and dispersibility was related to the concentrations of reactive thiol groups that determined the extent of cross linking during acidification. Yoghurt
microstructure was altered by whey protein ingredients, which significantly reduced void spaces and increased gel matrix compactness.

Bayarri et al. (2010) obtained information about how perceptible sensory differences affect consumer acceptability for yoghurt and a yoghurt-like product. Descriptive sensory profiles of six commercial samples, three of plain yoghurt and three of plain fermented milk, were determined using a trained panel (n=10). Sample acceptance was determined by a group of consumers (n=120). Initially, two groups of consumers were identified using Cluster analyses. For one group 46 (38%) of the consumers, variability in sensory attributes did not affect sample acceptability. For the second group, of 74 (62%) of the consumers, variability in sensory attributes had a significant effect and three consumer subgroups with different preference criteria were detected. Partial least squares regression was used to determine the sensory factors driving liking/disliking for each consumer subgroup. The information obtained can be important in predicting or explaining the market response to these types of products.

Xuesong (2010) reported that production of yoghurts via fermentation of milk partially substituted by soybean proteins was explored. Physical and chemical indexes, sensory indexes, texture parameters and amino acid composition of the yoghurts were fermented yoghurts were analyzed. Results showed that the texture parameters of yoghurt prepared with milk protein which was substituted by 50% soybean proteins, such as hardness, cohesiveness and adhesiveness, were lower than that by milk fermentation. Besides, yoghurt prepared with 50% soybean proteins had low acidity, amino acid score and some fishy smell, and yoghurt properties. This research suggested that soybean proteins could partially substitute milk protein without obvious decrease in the quality of yoghurt.

Wei et al. (2010) showed that stirred yoghurt was prepared using raw milk with different fat content and its viscosity, titrable acidity, pH, sensory quality and whey exhalation rate were determined. Results showed that, when the fat content in raw milk was less than 0.8%, the viscosity of the yoghurt was lower than 0.2 Pa.s and whey in the yoghurt was separated. The product also showed bad taste without milk flavour. With the increased and its sensory quality was also improved.

Xiujuan et al. (2010) showed the influence of different food gums on the stability of acidified milk with high protein content. The results showed that the best dosage of CMC-Na,
the ratio of xanthan gum to konjac gum, dosage of the mixture of xanthan gum to konjac gum and the dosage of Arabic gum were 0.4%, 3:2, 0.03%, and 0.02%, respectively, under which the product showed the highest stability, without fat separation and centrifugation precipitation.

Zamberlin et al. (2010) investigated the effect of heat treatment of ovine milk at 60°C/5 minutes and 90°C/5 minutes (control group) on the compositional and sensory properties of set yoghurt (n=40). The concentration of apparent casein and total whey protein were significantly higher while sensory properties (except consistency) were not significantly different from the yoghurts in control group (P<0.05). The results showed that ovine set yoghurt produced by heat treatment at low temperature possessed higher amount of preserved inherent functional and nutritional properties of milk than yoghurt produced by heat treatment at high temperature.

Sert et al. (2011) monitored the influence of sunflower honey addition (2%, 4% and 6% w/v) to yoghurt milk on survival of the microbial flora of yoghurt and the physicochemical and sensory characteristics during refrigerated storage for 4 weeks. The water activity decreased according to the action of honey with the higher concentration in the storage period (4°C). At the end of fermentation, pH values of yoghurt samples ranged between 4.33 (without honey) and 4.52 (addition of honey with 6%). The highest water holding capacity, consistency and the lowest brightness values were determined in the group produced with 6% honey addition. The water holding capacity and a*(redness) values of the honey incorporated yoghurt sample increased during storage. Streptococcus thermophilus and Lactobacillus delbrueckii subsp.bulgaricus values of the yoghurt with honey increased compared with the control group samples (P < 0.01). Addition of honey to yoghurt with has affected the vitality of the characteristics starters in the incubation and storage time of the yoghurt samples (P <0.01). Optimum sweetness was obtained with the samples containing 4% honey level.

Kim et al. (2011) examined the physiochemical, microbial, and sensory properties of yoghurt made by supplementing powdered yam Dioscorea opposita Thunb. (YPT) at different concentrations (0.2,0.4, 0.6 and 0.8 %, wt/vol) into milk, which was pasteurized and then fermented at 43 °C for 6 h and stored for 16 d. The pH values of all samples decreased, whereas viscosity values and mean microbial counts increased during storage. The L* and a* colour values (indicators of lightness and redness, respectively) of yoghurt samples were not remarkably influenced by adding YPT, whereas the b* values (indicating yellowness)
significantly increased with the addition of YPT at all concentrations at 0 d of storage, probably due to the original yellow colour of yam powder. In functional component analyses, when the concentration of YPT increased, the amount of allantoin and disogenin proportionally increased. The content of allantoin was 3.22 and disogenin 4.69 ug/mL when 0.2% (wt/vol) YPT was supplemented and did not change quantitatively during the storage period (16 d). The sensory test revealed that the overall acceptability scores of YPT-supplemented yoghurt samples (0.2 to 0.6%, wt/vol) of YPT could be used to produce YPT-supplemented yoghurt without significant adverse effects on physiochemical, microbial, and sensory properties, and enhance functional components from the supplementation.

2.1.8 Low-Fat/non-fat Yoghurt

*Skinner et al. (2003)* concluded that low-fat dairy products such as yogurt in their children’s healthy way of eating, given the rate at which childhood obesity is rising in the West: consumption of calcium-rich foods was found to be negatively correlated with body fat.

*Larsson et al. (2005)* indicated that enjoying full-fat yogurt and other full-fat dairy foods, such as whole milk, kefir, cheese, cream, sour cream and butter, may significantly reduce risk for colorectal cancer.

*Ripudaman (2005)* found that the consumption of low fat yoghurt could promote weight loss. In the trial, obese individuals who ate 3 servings of low fat yoghurt a day as part of a low calorie diet lost 22% more weight than the control group who only cut back on calories and did not have extra calcium. They also lost 81% more abdominal fat.

*Wood and Rebecca (2005)* reported that the nutritive value of low-fat yogurt for 1 cup or 245 grams.

### 2.1.8 Food Rating System Chart

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yogurt, 1.00 cup</td>
<td>245.00 grams</td>
</tr>
<tr>
<td>155.05 calories</td>
<td></td>
</tr>
</tbody>
</table>

low-fat

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yogurt, 1.00 cup</td>
<td>245.00 grams</td>
</tr>
<tr>
<td>155.05 calories</td>
<td></td>
</tr>
<tr>
<td>Nutrient</td>
<td>Value</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>iodine</td>
<td>87.22 mcg</td>
</tr>
<tr>
<td>calcium</td>
<td>447.37 mg</td>
</tr>
<tr>
<td>phosphorus</td>
<td>351.58 mg</td>
</tr>
<tr>
<td>vitamin B2 (riboflavin)</td>
<td>0.52 mg</td>
</tr>
<tr>
<td>protein</td>
<td>12.86 g</td>
</tr>
<tr>
<td>vitamin B12 (cobalamin)</td>
<td>1.38 mcg</td>
</tr>
<tr>
<td>tryptophan</td>
<td>0.06 g</td>
</tr>
<tr>
<td>potassium</td>
<td>572.81 mg</td>
</tr>
<tr>
<td>molybdenum</td>
<td>11.27 mcg</td>
</tr>
<tr>
<td>Zinc</td>
<td>2.18 mg</td>
</tr>
<tr>
<td>vitamin B5 (pantothenic acid)</td>
<td>1.45 mg</td>
</tr>
</tbody>
</table>

(Source: www.whfoods.com)

Reddy et al. (2007) studied to determine the effect of various factors upon B-vitamin content of cultured yogurt and to compare the B-vitamin contents of cultured and direct acidified yogurt. Incubation of yogurt culture at 42 C for 3 h yielded maximum vitamin synthesis concurrent with optimal flavour and texture qualities. A method was standardized for the manufacture of direct acidified yogurt involving the use of Stabilac acidulant and non-fat dry milk, Carboxymethyl cellulose, gelatin, and Starite. Acidified yogurt showed a slightly higher content of certain B-vitamins than the cultured yogurt due to the contribution made by various food additives. Both cultured and acidified yogurt showed good keeping quality and freedom from microbial contaminants during storage at 5 C for 16 days. However, folic acid and vitamin B12 contents decreased 29 and 60% in cultured yogurt and 48 and 54% in acidified yogurt.
Akin et al. (2009) evaluated reduced-fat stirred yoghurts that were prepared by using ropy and nonropy exopolysaccharide (EPS)-producing strains or nonexopolysaccharide-producing strains. Yoghurts were evaluated for pH, Titrable acidity, acetaldehyde, whey separation, viscosity, EPS concentration, sensory and microbial analyses. Determination of these parameter were made at 1, 7, 14 and 21 days of storage. Reduced-fat yoghurts made with EPS + strains exhibited lower syneresis and acetaldehyde contents, but higher viscosity than those made with the EPS − strain. Physico-chemical results correlated with the sensory results in that panellists scored the EPS + yoghurts as having an overall better mouth feel, but a worse flavour than EPS − yoghurt.

Abbasi et al. (2009) investigated the effects of different cultures and incubation temperatures on the physical properties of low fat yoghurts were investigated. The samples were incubated with exopolysaccharide (EPS) - producing and non-EPS-producing cultures at 37, 42 and 45°C. All measured parameters except firmness were influenced by culture type and incubation temperature. Firmness, G’ and G’’ were maximised at 42°C for both cultures. Increased incubation temperature and EPS culture led to a higher water-holding capacity but lower syneresis, G’ and G’’. The EPS treatment incubated at 37°C showed even lower syneresis than non-EPS treatments incubated at higher temperatures.

Hanson and Metzger (2010) studied the effect of increased vitamin D fortification (250 IU/serving) of high-temperature, short-time (HTST)-processed 2% fat milk. UHT-processed 2% fat chocolate milk and low-fat strawberry yoghurt on the sensory characteristics and stability of vitamin D during processing and storage. Three replicates of HTST pasteurized 2% fat milk, UHT pasteurized 2% fat chocolate milk, and low-fat strawberry yoghurt were manufactured. Each of 3 replicates for all products contained a control (no vitamin D fortification), a treatment group with 100 IU vitamin D/serving (current level of vitamin D fortification), and a treatment group with 250 IU vitamin D/serving. A cold-water dispersible vitamin D3 concentrate was used for all fortifications. The HTST-processed 2% fat milk was stored for 60 d, with vitamin D analysis done before processing and on d 0, 40 and 60. Sensory analysis was conducted on d 40. Low-fat strawberry yoghurt was stored for 42d, with vitamin D analysis done before processing, and on d 0, 28 and 42. Sensory analysis was conducted on d 28. Vitamin D levels in the fortified products were found to be similar to the target levels of fortification (100 and 250 IU vitamin D/serving) for all products, indicating no loss of vitamin D during processing. Vitamin D was also found to be stable over the shelf
life of each product. Increasing the fortification of vitamin D from 100 to 250 IU/serving did not result in a change in the sensory characteristics of HTST-processed 2% fat chocolate milk or low-fat strawberry yoghurt. These results indicate that it is feasible to increase vitamin D fortification from 100 to 250 IU per serving in these products.

Sivakumar et al. (2010) evaluated the effect of different levels of soy protein isolate (SPI; 0, 0.5, 1.0 and 1.5%) and different levels of carrot juice (5, 10 and 15%) along with/without optimized level of SPI (1%) on low-fat sweetened dahi (Indian yoghurt) was evaluated. Titratable acidity and pH values varied in narrow range without any significant difference with different treatments of SPI incorporation. However, fat content decreased and protein, ash, carbohydrate and total solids increased significantly (p<0.05). Further, titratable acidity increased and pH decreased with increased levels of carrot juice. But, fat protein, total solids and ash decreased significantly (p<0.05). Textural parameters differ significantly (p<0.05) with different treatments due to variations in the composition. Instrumental colour evaluation indicated decreased L value, increased a-value due to incorporation of SPI and carrot juice. It is concluded that 10% carrot juice along with 1% Spi improves the sensory, physicochemical properties of low fat sweetened dahi.

Michael et al. (2010) studied the effect of a plant extract (prepared from olive, garlic, onion and citrus with sodium acetate as a carrier) on the viability of yogurt starter cultures. Non-fat yogurt was prepared with various levels of supplements: plant extract (0, 0.5 or 1.0%, w/v) or l-cysteine HCL (0.014 or 0.028%, w/w). Microbial and physicochemical analyses were conducted weekly for 50 days. Fermentation time increased for supplemented yoghurts compared with the non-supplemented yoghurt. Lactobacillus bulgaricus counts in supplemented yoghurts were >6 log cfu ml⁻¹ for a longer time (7-21 days) compared with the non-supplemented yoghurt. Streptococcus thermophilus counts in all yoghurts were >6 log cfu ml⁻¹ throughout the storage. Overall, redox potential and titratable acidity of yoghurts on day 50 were greater compared with day 1, but pH and syneresis were less. Plant extract at 0.5% enhanced L. bulgaricus viability in non-fat yoghurt while least affecting the physicochemical characteristics.

Maowen et al. (2010) showed that the mixed juice of pumpkins and apples was fermented by using yeast and optimum fermentation conditions for pumpkins-apples wine production were determined as follows: ratio of 8%, fermentation time 3 days, and the sugar degree 8 Brix. Then the milk was mixed with stabilizing agents and sugar and inoculation with lactic acid
bacteria. And the best conditions for stirred yoghurt production were as follows: stabilizing agent’s content of 0.25%, modified starch-sodium alginate-pectin ratio of 2:2:0.5, and the homogenization temperature of 70°C. Then, 10% fruit wine was added to the stirred yoghurt, foaming a new kinds of wine-flavoured stirred yoghurt.

Nancy et al., 2010 reported the effect of a prebiotic (fructooligosaccharide) or a synbiotic ingredient (fructooligosaccharide and Lactobacillus acidophilus) on the sensory properties and consumer acceptability of peach-flavored drinkable yogurts. Descriptive analysis and consumer testing were carried out for the six yogurt drinks used in this study. An analysis of variance (ANOVA) of the descriptive data showed significant differences ($P < 0.05$) among the samples for 8 of the 12 attributes. Both the descriptive and consumer data indicated that the differences among the samples were either because of the fat content or the presence of synbiotics and prebiotics. The yogurts containing the prebiotic were not significantly different from their comparable controls indicating that a prebiotic can be added without impacting acceptance. However, the samples (skim milk and whole milk) containing the synbiotic were the least acceptable indicating that synbiotic combination had a negative impact on acceptance.

Martin et al. (2011) investigated the effect of oxidoreduction potential (EH) on the biosynthesis of aroma compounds by lactic acid bacteria in non-fat yoghurt. The study was done with yoghurts fermented by Lactobacillus bulgaricus and Streptococcus thermophilus. The Eh was modified by the application of different gaseous conditions (air, nitrogen, and nitrogen/hydrogen). Acetaldehyde, dimethyl sulphide, diacetyl and pentane-2, 3-dione, as the major endogenous odorant compounds of yoghurt, were chosen as tracers for the biosynthesis of aroma compounds by lactic acid bacteria. Oxidative conditions favoured the production of acetaldehyde, dimethyl sulphide, and diketones (diacetyl and pentane-2, 3-dione). The Eh of the medium influences aroma production in yoghurt by modifying the metabolic pathways of Lb. bulgaricus and Streptococcus thermophilus. The use of effect of oxidoreduction potential as a control parameter during yoghurt production could permit the control of aroma formation.

Darclee and Zeynep 2011 reported that Low-fat strawberry yoghurts were prepared with each of five alternative sweeteners: sucrose, high-fructose corn syrup and honey from three different floral sources. A ninety-nine-member consumer panel evaluated the yoghurts for flavour, aroma, sweetness and overall acceptance. Degree of liking was scored by the
Panellists on a nine-point hedonic scale. Panellists preferred sucrose-sweetened yoghurts over those sweetened with high-fructose corn syrup and honey ($P < 0.05$). Among the honey-sweetened yoghurts, sage honey was the most liked ($P < 0.05$), followed by alfalfa- and sourwood-sweetened yoghurts.

2.1.9 Yoghurt as carrier of probiotics:

Shah 2001 showed that application of probiotics as a starter culture is limited because it takes more incubation period and the product quality may be affected with respect to texture and flavour. Thus, the normal practice is to use probiotics as adjunct starters. Yoghurts containing *L. acidophilus* and *Bifidobacteria* are termed ‘AB’ yoghurts &those containing *L. casei* in addition to the above are termed ‘ABC’ yoghurt.

2.2 Frozen Yoghurt

2.2.1 Different Composition for Manufacturing Frozen Yoghurt

Hekmat and McMahon 2002 stated that probiotic ice cream was made by fermenting a standard ice cream mix with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* cultures and then freezing the mix in a batch freezer. Survival of the *L. acidophilus* and *B. bifidum*, as well as beta-galactosidase activity, was monitored during 17 week of frozen storage at -29 degrees C. After freezing of the fermented mix, bacterial counts were $1.5 \times 10^8$ cfu/ml for *L. acidophilus* and $2.5 \times 10^8$ cfu/ml for *B. bifidum*. Seventeen weeks after freezing, these counts had decreased to $4 \times 10^6$ and $1 \times 10^7$ cfu/ml, respectively. During the same period, beta-galactosidase activity decreased from 1800 to 1300 units/ml. Probiotic ice cream was prepared at pH 5.0, 5.5, and 6.0 to determine consumer preferences and was compared with standard Utah State University "Aggie" ice cream. All samples were strawberry-flavoured and were evaluated by 88 judges. The preferred pH of probiotic ice cream, based on overall acceptance, was pH 5.5. We demonstrated that probiotic ice cream is 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 ice cream mix and remain viable during frozen storage.

Martini et al., 2007 found that lactose digestion from and tolerance to flavoured and frozen yogurts, ice cream, and ice milk were evaluated (20 g lactose/meal) in lactase-deficient subjects by use of breath hydrogen techniques. Unflavoured yogurt caused significantly less hydrogen production than milk (37 vs 185 delta ppm X hr, n = 9).
Flavoured yogurt was intermediate (77 delta ppm X hr). Subjects were free of symptoms after consuming flavoured and unflavoured yogurts. Of seven commercial yogurts tested, all contained significant levels of microbial beta-galactosidase (beta-gal). In addition, eight subjects were fed meals of milk, ice milk, ice cream, and frozen yogurts with and without cultures containing high levels of beta-gal. Peak hydrogen excretion after consumption of frozen yogurt with high beta-gal was less than one-half of that observed after the other five test meals and intolerance symptoms were absent. Tolerance to frozen yogurt, produced under usual commercial procedures, was found to be similar to that of ice milk and ice cream.

Isik et al. 2011 reported on frozen yogurt containing low fat and no added sugar. Samples containing 5% polydextrose, 0.065% aspartame and acesulfame-K mixture, and different levels of inulin and isomalt (5.0, 6.5, and 8.0%) were produced at pilot scale and analyzed for their physical and chemical properties including proximate composition, viscosity, acidity, overrun, melting rate, heat shock stability, as well as sensory characteristics, and viability of lactic acid bacteria. With the addition of inulin and isomalt, viscosity increased by 19 to 52% compared with that of sample B (reduced-fat control). The average calorie values of samples substituted with sweeteners were about 43% lower than that of original sample. Low-calorie frozen yogurt samples melted about 33 to 48% slower than the reduced-fat control sample at 45 min. Based on quantitative descriptive profile test results, statistically significant differences among products were observed for hardness, iciness, foamy melting, whey separation, and sweetness characteristics. The results of principal component analysis showed that the sensory properties of the sample containing 6.5% inulin and 6.5% isomalt were similar to those of control. Lactic acid bacteria counts of frozen yogurt were found to be between 8.12 and 8.49 log values, 3 mo after the production. The overall results showed that it is possible to produce an attractive frozen yogurt product with the incorporation of inulin and isomalt with no added sugar and reduced fat.

### 2.2.2 Suggested Chemical Composition (g/100g) for Frozen Yoghurt:

<table>
<thead>
<tr>
<th>Ingredients</th>
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<tbody>
<tr>
<td></td>
<td>Soft</td>
</tr>
<tr>
<td>Fat</td>
<td>2-6</td>
</tr>
<tr>
<td>Component</td>
<td>5-10</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Milk SNF</td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
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<tr>
<td>Stabilizer/emulsifier</td>
<td>1.2-1.0</td>
</tr>
<tr>
<td>% over-run</td>
<td>50-60</td>
</tr>
</tbody>
</table>

(Source: Tamine et al. 1883)

Turgut and Cakmakci (2009) investigated the possibility of using some types of probiotic bacteria in the production of ice cream and were aimed at making a contribution to the manufacture of new functional foods. For this purpose, different cream levels (5% and 10%) and different strains of probiotic bacteria (Lactobacillus acidophilus, bifidobacterium bifidum and both) were used in ice cream production to determine their effects on the quality of the ice creams in each group. During storage of 1, 15, 30, 45, 60, 75 and 90 days, L. acidophilus, B. bifidum counts and sensory analyses were performed. The results obtained at the end of storage demonstrated that the counts of L. acidophilus and B. bifidum continued to decrease during the storage but all types of ice cream sample seemed to preserve their probiotic property even after 90 days. Higher counts of probiotic bacteria were observed in the sample with L. acidophilus and B. bifidum in double-cultured samples respectively. In general, it appeared that the ice cream samples with 5% cream content were found to be more delectable. All ice cream samples have shown good results in preserving their probiotic properties for more than 3 months. Although sensory scores of probiotic ice cream samples reduced during this time, they rated as ‘tasty’ throughout the storage.

Milani and Koocheki (2011) examined the effect of adding date syrup (0%, 25% and 50% as sugar replacement) and guar gum (0%, 0.1%, 0.2% and 0.3% as fat replacement) in respect of physicochemical, rheological and sensory properties of low fat frozen yoghurt. Increasing date syrup and guar gum concentration increased the mixture viscosity and acidity. Guar gum softened the frozen yoghurt whereas date syrup increased the hardness of the product. The control yoghurt was less sticky than samples containing the gum, but date syrup had no significant effect on the samples stickiness. Melt-down stability of the samples also increased with increase in gum and date syrup concentration. The low fat orange flavoured frozen yoghurt dessert prepared in this study also had good sensory properties.
Nousia et al. (2011) stated that probiotic ice cream was produced by incorporating *Lactobacillus acidophilus* LMGP-21381 in a standard ice cream mix at initial population above $10^7$ cfu/g. The ice cream mix was inoculated with either freeze-dried or activated cultures of *L. acidophilus* and a control treatment without probiotic was also prepared. The product was assessed for the survival of the probiotic strain during the freezing process and during 45 weeks of storage at -15°C and -25°C, and also for its sensory characteristics. The results showed that the freezing process caused a significant decrease in the viability of the freeze−dried culture, but no significant change in the viable counts of *L. acidophilus* was observed during frozen storage. The sensory attributes of aroma, taste and texture obtained high scores in the sensory evaluation. It was demonstrated that incorporation of either activated or commercial freeze-dried *L. acidophilus* culture resulted in a candidate food for the delivery of high levels of this probiotic strain to consumers.

### 2.2.3 Health Benefits of Frozen Yoghurt with probiotic culture

Corte (2008) stated that the search for the population’s better quality of life is attracting the food industry interest in developing products with functional characteristics, offering whole foods, fortified, enriched or improved food, causing effects potentially beneficial to healthy in the preventive and therapeutic aspects. This work aimed to elaborate frozen yoghurt with functional properties from yoghurt supplemented with probiotic (inulin), calcium caseinate and probiotics. The lactic cultures used were the traditional and the probiotic lactic cultures in three different concentrations (0.5%, 1.0%, 1.5%). The elaboration process of the frozen occurred from the development of the yoghurt, in concentration already mentioned, observing the pH values and later, through the processes of homogenization of the ingredients, mixing in low temperature (ice cream maker), packing and freezing of the product at a temperature of -22°C. The carried out physicochemical analyses were for pH, acidity expressed as lactic acid, lactose level, ash level, protein level, fat level and overrun; the microbiological analysis, in a 35-day period of storage and in 7-day intervals, to evaluate the feasibility of the traditional species lactic cultures *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *Bulgaricus* and probiotics *Lactobacillus acidophilus* and *Bifidobacterium*. Microbiological analyses of the traditional lactic cultures and biofilized probiotics were carried out for the certification of the number of microorganisms, respectively. Sensorial analysis was carried out in three alternate days, with two days in the UNIFRA (Franciscan University of Santa Maria, RS) Technical and Dietetic Laboratory and the third day in the UFSM (Federal University of Santa Maria, RS) Sensorial
Analysis Laboratory with the analyzed features being: colour, flavour, smell, consistency and total quality using a structured hedonic scale with 9 scores. The intention of buying the product and its preference were evaluated in this research making 105 non-trained tasters. The experimental data generated through the sensorial analysis results were submitted to variance analysis (ANOVA) and the differences of the means compared through the Tukey. Concentrations of 1.0% and 1.5% of traditional cultures and probiotic had no significant difference in relation to sensory analysis developed. To order the test was used in preference to the table and Newell MacFarlane where the formulation of 0.5% of crops and traditional probiotics milk obtained the lower acceptability.

Davidson et al. (2000) reviewed that low-fat ice cream mix was fermented with probiotic supplemented and traditional starter culture systems and evaluated for culture survival, composition, and sensory characteristics of frozen product. Fermentations were stopped when the titratable acidity reached 0.15% greater than the initial titratable acidity (end point 1) or when the pH reached 5.6 (end point 2). Mix was frozen and stored for 11 week at –20°C. The traditional yogurt culture system contained the strains Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. The probiotic-supplemented system contained the traditional cultures as well as Bifidobacterium longum and Lactobacillus acidophilus. We compared recovery of Bifidobacterium by three methods, a repair-detection system with rolltubes and plates on modified bifid glucose medium and plates with maltose + galactose reinforced clostridial medium. Culture bacteria in both systems did not decrease in the yogurt during frozen storage. The roll-tube method with modified bifid glucose agar and repair detection system provided at least one-half log10 cfu/ml higher recovery of B. longum compared with recoveries using modified bifid glucose agar or maltose + galactose reinforced clostridial agar on petri plates. No change in concentrations of lactose or protein for products fermented with either culture system occurred during storage. Acid flavor was more intense when product was fermented to pH 5.6, but yogurt flavor was not intensified. The presence of probiotic bacteria in the supplemented system seemed to cause no differences in protein and lactose concentration and sensory characteristics.

Kartz (2001) reported that yoghurt was introduced to the American diet during the 1940s. By the 1980s, it had become the product for dieters and the lunch of choice for young women. The use of yoghurt as a calcium source has made it one of the most rapidly growing dairy products, but presently it is more than just a calcium source. Yogurt, kefir, and similar
fermented milk products are on the way to becoming major nutraceuticals aimed at treating a variety of disease conditions

2.3 Probiotic Yoghurt

2.3.1 Definition of probiotic:

According to the World Health Organization (WHO) probiotics are defined as “Live microorganisms which when administered in adequate amounts confer a health benefit to the host (Brown et al., 2005, Adhikari et al., 2000, Sanchez et al., 2009)

2.3.2 Benefit of Probiotic Strains in Yoghurt

Vinderola et al., (2000) reported that they evaluated the suitability of Argentinian Fresco cheese as a food carrier of probiotic cultures. They used cultures of Bifidobacterium bifidum (two strains), Bifidobacterium sp. (one strain), Lactobacillus acidophilus (two strains), and Lactobacillus casei (two strains) in different combinations, as probiotic adjuncts. Probiotic, lactic starter (Lactococcus lactis and Streptococcus thermophilus), and contaminant (coliforms, yeasts and moulds) organisms were counted at 0, 30, and 60 d of refrigerated storage. Furthermore, the acid resistance of probiotic and starter bacteria was determined from hydrochloric solutions (pH 2 and 3) of Fresco cheese. The results showed that nine different combinations of bifidobacteria and L. acidophilus had a satisfactory viability (count decreases in 60 d 1log order) in the cheese. Both combinations of bifidobacteria and L. casei cultures assayed also showed a satisfactory survival (counts decreased 1 log order for bifidobacteria but no decrease was detected for L. casei). On the other hand, the three combinations of bifidobacteria, L. acidophilus, and L. casei tested adapted well to the Fresco cheese environment. When a cheese homogenate at pH 3 was used to partially simulate the acidic conditions in the stomach, the probiotic cultures had an excellent ability to remain viable up to 3 h. At pH 2, the cell viability was more affected; B. bifidum was the most resistant organism. This study shows that the Argentinian Fresco cheese could be used as an adequate carrier of probiotic bacteria.

Ekinici and Gurel (2008) suggested that propionibacteria are able to produce a wide variety of food components beneficial to human health. In this study, yoghurt was produced by using the adjunct starter cultures Propionibacterium jensenii B1264 and Propionibacterium thoenii (jensenii) P126. Although the total solids and protein contents of the yoghurts did not show
any significant differences, titratable acidity of the control sample (YC-380) remained lower than that of *Propionibacterium* spp. supplemented yoghurts during 15 days of storage. The yoghurts produced by YC-380 + P126 cultures had the firmest structure (0.26 N). The highest acetaldehyde (29.35 mg/kg) content was obtained with yoghurt made with YC-380 + P126 + B1264 on d 1. The addition of propionibacteria to yoghurt did not have any negative effect on the counts of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus* in yoghurt. During the first week of storage, *propionibacteria* counts remained high, suggesting that yoghurt provided a good environment for these organisms. This new product would provide not only beneficial health effects, but also a new alternative product to plain set type yoghurt.

**Kearney et al. (2009)** monitored that the viability of probiotic *Lactobacillus paracasei* NFBC 338 during: (a) two-stage yoghurt fermentation with starter cultures *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (b) following spray drying and (c) during storage for 42 days. During the initial fermentation phase (10h), probiotic lactobacillus numbers increased 7-fold to $3.9 \times 10^9$ cfu/g and these numbers were maintained during fermentation for a further 3 h in the presence of the yoghurt starters. Following spray-drying, the probiotic culture survived best, followed by *S. Thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (yielding $3.4 \times 10^8$, $1.2 \times 10^8$ and $4.0 \times 10^5$ cfu g$^{-1}$ powders, respectively). *L. paracasei* NFBC 338 and *S. thermophilus* were stable during storage at 4°C and 15°C (for 42 days) with viable counts exceeding $10^7$ cfu/g$^{-1}$, while viability of *L. delbrueckii* subsp. *bulgaricus* decreased considerably throughout storage.

**Patil et al. (2009)** investigated the optimum level of guava (*Psidium guajava* L.) pulp that could be incorporated to obtain the best quality guava yoghurt with improved flavor, body and texture was investigated. Addition of guava pulp at 5-15% after incubation in the stirred form with addition of 6-9% sugar was more acceptable. Guava yoghurt prepared with 5 and 9% sugar was sensorily superior to that of control sample. The final product contained 3.4% fat, 21.5% total solids, 1.1% acidity, 5.0% reducing sugar, 7.9% non-reducing sugar, 13.5% total sugar with pH 4.4.

**Kudejka (2010)** analyzed that the survival rate of probiotic bacteria in bio-yoghurts, manufactured of goat’s milk, during their refrigerating storage. While storing natural bio-yoghurts of goat’s milk, the count of probiotic bacteria decreased. Changes in the count of *Bifidobacterium bifidum* and of *Streptococcus thermophilus* bio-yoghurts of goat’s milk
were statistically insignificant. Changes in the count of *L. acidophilus* cells in bio-yoghurts of goat’s milk being stored were statistically significant, but, during a 21 day period of storage, their count increased and was higher than 10⁶ cfu/ml. Moreover, a high survival rate of probiotic bacteria was evidenced, and this fact means that it is possible to manufacture drinks showing high healthy values from goat’s milk.

### 2.3.3 Health benefits of Probiotic Yoghurt

Siitonen (2000) reported that “Let food be thy medicine and medicine be thy food,” the age-old quote by Hippocrates, is certainly the tenet of today. With the growing interest in self-care and integrative medicine coupled with our health embracing baby boomer population, recognition of the link between diet and health has never been stronger. As a result, the market for functional foods, or foods that promote health beyond providing basic nutrition, is flourishing. Within the functional foods movement is the small but rapidly expanding arena of probiotics – live microbial food supplements that beneficially affect an individual by improving intestinal microbial balance. The consumers’ overwhelming interest in and demand for functional foods, including probiotics, make it imperative that health professionals stay abreast of the latest research findings and available products. This monograph provides a summary of research on the health benefits of probiotics and offers practical information to help the clinician make appropriate recommendations to clients.

Sinha and Sinha (2000) showed that fermented milks are known throughout the world for their taste, nutritive values and therapeutic properties. Preservation of milk solids in the form of fermented milk has been considered very simple & immediately accessible convenient method. Several countries have their own specific fermented milk.

Branca and Rossi (2002) reported that probiotic bacteria are used for production of fermented dairy products. The use of probiotic bacteria has the potential to replenish the natural intestinal flora of the body. These bacteria competitively inhibit the growth and colonization of pathogenic bacteria. Breast milk is the best food for babies, also from a probiotic point of view. Human milk, in fact, contains many substances that stimulate the growth of bifidobacteria in vitro and in the small intestine of infants. Improvement of lactose digestion and avoidance of symptoms of intolerance in lactose malabsorbers are the most profoundly studied health-relevant effects of fermented milk. In fact fermented milks are nutritionally similar to unfermented milk, except that some of lactose is broken down to
glucose and galactose. The role of fermented milk in complementary feeding and in particular for the prevention of anaemia is an innovative theme, recently focused. Iron deficiency in infants and young children is widespread and has serious consequences for child health. Prevention of iron deficiency should therefore be given high priority. The too-early introduction of unmodified cow’s milk and milk products is important nutritional risk factors for the development of iron deficiency anaemia. Fermented milks represent an excellent source of nutrients such as calcium, protein, phosphorus and riboflavin. During the fermentation of milk, lactic acid and other organic acids are produced and these increase the absorption of iron. If fermented milk is consumed at mealtimes, these acids are likely to have a positive effect on the absorption of iron from other foods.

Baharav et al. (2004) studied that Lactobacillus, a probiotic (friendly) bacteria found in yogurt offers "remarkable preventive and curative" effects on arthritis. Lactobacillus has already demonstrated beneficial effects in other inflammatory diseases such as inflammatory bowel disorders, researchers thought it might also lessen the inflammation of arthritis. To find out, they ran two groups of animal experiments. In both sets of experiments, laboratory animals fed the yogurt with large amounts of lactobacilli had the least amount of arthritic inflammation, while those fed plain yogurt experienced only moderate inflammation. The animals that received just lactobacillus, even heat-killed lactobacillus, also showed significant benefit. Milk, however, had no effect. So impressed were the researchers with the study's results that they recommended trials using commercial yogurts containing lactobacilli in arthritic patients.

Suvarna and Boby (2005) showed that microbial cultures have been used for thousands of years in food and alcoholic fermentations, and in the past century have undergone scientific scrutiny for their ability to prevent and cure a variety of diseases. This has led to the coining of the term probiotics. Today probiotics are available in a variety of food products and supplements, and have got wide applications in the control of cholesterol, cancers, allergies, etc. This article discusses the summary of research on the health benefits of probiotics and offers practical information to help health professionals and even the layman.

Villena (2005) studied on laboratory animals were fed a balanced conventional diet with or without supplemental lactobacillus casei for 7, 14 or 21 days, then challenged with S. pneumoniae. In all groups of animals given lactobacillus casei, normalization of the immune response and recovery occurred much more quickly than in controls, who received only the
balanced conventional diet. Controls took 21 days to regain a normal immune response, but test animals fed the friendly bacteria recovered normal immunity in just 7 days! In addition, malnourished mice receiving \textit{lactobacillus casei} were able to more effectively clear the pneumonia pathogen from their blood and had significantly less lung damage than controls. A human study has confirmed that a daily serving of probiotic-rich yogurt bolsters your body's ability to protect you from infection.

\textbf{Heller (2008)} showed that probiotic bacteria are sold mainly in fermented foods, and dairy products play a predominant role as carriers of probiotics. These foods are well suited to promoting the positive health image of probiotics for several reasons: 1) fermented foods and dairy products in particular, already have a positive health image; 2) consumers are familiar with the fact that fermented foods contain living microorganisms (bacteria); and 3) probiotics used as starter organisms combine the positive images of fermentation and probiotic cultures. When probiotics are added to fermented foods, several factors must be considered that may influence the ability of the probiotics to survive in the product and become active when entering the consumer's gastrointestinal tract. These factors include 1) the physiologic state of the probiotic organisms added (whether the cells are from the logarithmic or the stationary growth phase), 2) the physical conditions of product storage (eg, temperature), 3) the chemical composition of the product to which the probiotics are added (eg, acidity, available carbohydrate content, nitrogen sources, mineral content, water activity, and oxygen content), and 4) possible interactions of the probiotics with the starter cultures (eg, bacteriocin production, antagonism, and synergism). The interactions of probiotics with either the food matrix or the starter culture may be even more intensive when probiotics are used as a component of the starter culture. Some of these aspects are discussed in this article, with an emphasis on dairy products such as milk, yogurt, and cheese.

\textbf{Tabatabaie and Mortazavi (2008)} reported that dairy products containing lactic acid bacteria are often consumed because of the health-promoting activities of some strains of these bacteria as probiotics. It is important that these strains survive the acidic environment of the product since only microorganisms that are alive can be functionally active. This report describes the potential of lactulose to improve the survival of available probiotic strains in yoghurt. Yoghurt with and without lactulose were produced in which these strains were present. The survival of the probiotic strains was monitored for 5 weeks at 4°C. The main conclusions are: \textit{Lactobacillus rhamnosus} and \textit{Bifidobacterium bifidum} were found to be
extremely stable during the 5 week storage period and survives slightly better the presence of lactulose.

Galdeano et al. (2009) studied that the intestinal ecosystem contains a normal microbiota, non-immune cells and immune cells associated with the intestinal mucosa. The mechanisms involved in the modulation of the gut immune system by probiotics are not yet completely understood. The present work studies the effect of fermented milk containing probiotic bacterium Lactobacillus (Lb.) casei Dn114001 on different parameters of the gut immune system involved with the nonspecific, innate and adaptive response. BALB/c mice received the probiotic bacterium Lb. casei DN114001 or the probiotic fermented milk (PFM). The interaction of the probiotic bacteria with the intestine was studied by electron and fluorescence microscopy. The immunological parameters were studied in the intestinal tissue and in the supernatant of intestinal cells (IC). Results showed that the probiotic bacterium interact with the IC. The whole bacterium or its fragments make contact with the gut associated immune cells. The PFM stimulated the IC with IL-6 release, as well as cells related to the nonspecific barrier and with the immune cells associate with the gut. This last activity was observed through the increase in the population of different immune cells: T lymphocytes and IgA+ B lymphocytes, and by the expression of cell markers related to both innate and adaptive response (macrophages). PFM was also able to activate the enzyme calcineurine responsible for the activation of the transcriptional factor NFAT. PFM induced mucosal immune stimulation reinforcing the non-specific barrier and modulating the innate immune response in the gut, maintaining the intestinal homeostasis.

Santo et al. (2010) demonstrated that the effects of acai pulp addition and different probiotic bacteria on the fatty acid profile of stirred yoghurt were examined. Skim milk was divided into two groups: one containing acai pulp and another without the fruit. Batches were inoculated with yoghurt starter culture and divided into five groups according to probiotic addition. Counts of viable microorganisms were measured at days 1, 14 and 28 of cold storage. Fatty acid profile was determined by gas chromatography at day 1. Acai pulp favoured an increase in Lactobacillus acidophilus L10, Bifidobacterium animalis ssp. lactis BI04 and Bifidobacterium longum BI05 counts at the end of 4 weeks of cold storage. This study demonstrated that acai pulp addition increased monounsaturated and polyunsaturated fatty acid contents in probiotic yoghurt and enhanced the production of α-linolenic and
conjugated linoleic acids during fermentation of skim milk prepared with *B. animalis ssp. lactis* B104 and B94 strains.

Allgeyer *et al.* (2010) assessed that the popularity of dairy products fortified with prebiotics and probiotics continues to increase as consumer’s desire flavourful foods that will fulfill their health needs. Our objectives were to assess the sensory profile of drinkable yoghurt made with prebiotics and probiotics and to determine the viability of the probiotics in the yoghurt drink over the duration of storage. Thirteen trained descriptive panelist evaluated 10 yoghurt drinks on a 16-point category scale. Three selected prebiotics, soluble corn fiber, polydextrose and chicory inulin, were each present individually at an amount to claim an excellent source of fiber (5 g of fiber/serving) or a good source of fiber (2.5 g of fiber/serving) in 6 different yoghurt drinks. Three additional yoghurt drinks contained 5 g of each of the separate probiotics along with a mixture of the selected probiotics (*Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* LA-5). A control sample with no prebiotics or probiotics was also included in the experimental design. Data were analyzed by ANOVA, Fisher’s least significant difference, and principle components analysis. Survival of the probiotics in the yoghurt drinks during a 30-d refrigerated storage period was also analysis. Results showed that clover honey aroma, buttermilk aroma, butter aroma, sweetness, sourness, chalky mouth feel, and viscosity were identified as significant attributes in the yoghurt drinks. Total variance explained by the principal component analysis bipot of factors 1 and 2 was 65%, which showed yoghurt drinks with soluble corn fiber and inulin varying by the sweet versus sour attributes and yoghurt drinks with the polydextrose varying by the mouthfeel attributes. The viability study determined a 2- to 3-log decrease in the survival of probiotics in all of the yoghurt treatments during a 30-days refrigerated storage period. Based on the results of the current study, only the polydextrose treatment would be an acceptable vehicle to deliver the probiotics health effects at the 30-days storage period.

**2.4 Carrot:**

**2.4.1 Merits of Carrots**

Barrett (1998) reported that carrot is the bright star of earth root vegetables nature’s neon orange garden spike loaded with vitamin A. Carrots are one of the few vegetables that are better for you cooked than raw (the cooking process makes the nutrients more readily available).
Robertson *et al.* (1999) said that two hundred grams of raw carrots eaten at breakfast each for 3 weeks, significantly reduced serum cholesterol by 11 percent, increased faecal bile acid and fat excretion by 50 percent and modestly increased stool weight by 25 percent. This suggests an associated change in bacterial flora or metabolism. The changes in serum cholesterol, faecal bile acids, and fat persisted 3 weeks after stopping treatment.

Robertson (2000) reported that a carrot is a renaissance vegetable that is consummate vegetable for all seasons. We grate carrots into summer salads, slice them into our springtime stir-fries, chop them into autumn soups and stews and roast them whole in the oven on cold winter days.

Baybutt *et al.* (2000) showed that vitamin A’s protective effects may help explain why some smokers do not develop emphysema. If you or someone you love smokes, or if your work necessitates exposure to second hand smoke, protect yourself by making sure the world’s healthiest foods rich in vitamin A, (carrot’s beta carotene is converted in the body into vitamin A) are a daily part of your healthy way of eating.

Digitalline (2001) said that the health benefits of carrot include reduced cholesterol, prevention from heart attacks, warding of certain cancers and many others. Carrots are rich in vitamin A, C, K and potassium. Both adults and children like carrots because of its crunchy texture and sweet taste. Even through the colour of original carrot is orange it grows in other colours including white, yellow, red or purple.

Vinodgm (2001) said that carrots are ideal for those intending to lose weight and also those who are health conscious. They are very easily eaten and tasty in their raw form. Carrots were formally in colours of red, black, yellow, white and more commonly purple. They also contain only a small amount of vitamin C equivalent to only about 10 percent of recommended daily allowances. The main vitamin in the carrot is vitamin A.

Gisha (2001) reported that carrots have antiseptic qualities and therefore, can be used as laxative, vermicide and as remedy for liver conditions. Carrot oil is good for dry skin. It makes the skin softer, smoother and firmer. Carrot juice improves stomach and gastrointestinal health. Thus, carrots, as raw fruits juices or in cooked form, are good for your health.

Marker (2002) stated that carrot is an excellent source of vitamin B and C. It contains 1890ug per 100g carotene. It contains calcium pectate, which has been reported to lower the
cholesterol level in cardiovascular disease, carrots contain the nutrients, which are helpful in fighting against diseases like certain types of cancers e.g. Gastrointestinal, mouth and lung. It also helps in fighting against ulcer too. Vitamin A content of carrot helps in keeping the skin healthy as vitamin A keeps cell membrane healthy, making them stronger against disease causing microorganisms.

Adams (2005) stated that carrots have long been believed to help keep eye sight strong, and help keep you cancer free. Natural pesticide found in carrots can stop cancer cells from becoming malignant. Organic carrots, has begun recommending everyone eaten at least one small carrot a day.

Micksheff (2006) reported that carrots are a biennial plant, taking two years to complete its life cycle. We interrupt this cycle by pulling up the plant after the first year and eating its fleshy root, if left in the ground the plant will flower the following spring and seed before dying. There are hundreds of different varieties of carrots the most popular from sold in our supermarkets today is known as the “Mediterranean carrot” which has a long and thin shape and a deep rich orange colour.

Magee (2006) stated that carrot a day may help keep a whole assortment of disease away. While we all known carrots are a great source of beta carotene, what we really should known is the best way to get your carotenes (carotenoids) is in a nature made assortment of this wonderful phytochemical sub family (including alpha carotene, beta carotene, gamma carotene, lutein, lycopene).

Alexis (2006) reported that carrots really do give your eyes a boost because they contain beta carotene, which the body is able to convert into vitamin A, an essential vitamin or healthy vision.

Elena (2007) concluded that carrots are a very good source of beta carotene. Food processors have found the importance of beta carotene not only as a precursor of vitamin A but also as an agent that prevents cancer. Vitamin A and beta carotene are popularly known to fight cancer and increase the body’s ability to ward off infection and recover quickly from illness.

James (2008) found that carrot juice is a miracle juice, or the king of juices. If Carrot juice is added in their daily diet, their overall health improves extremely. A common misconception is that carrot juice is bitter. You can also use carrot juice to lose weight by adding celery
while juicing. Carrot juice is best ingested in the afternoon because of the burst of energy that always flows from it.

Dolores (2008) found that antioxidants like beta carotene sponge up free radicals, those unstable molecules that cause many of the ills of advancing years. Among their many benefits, carrots are also noted for helping to prevent cataracts. Never be without them in your refrigerator. A whole medium carrot is only 30 calories.

Gutierrez (2008) found that carrots contain naturally occurring calcium, the mineral is poorly absorbed by the human body. In the modified carrots, a gene has been changed to allow calcium to move more freely across the carrots cell membranes, absorb 41 percent more calcium from the genetically modified carrot than from the natural variety. That amounts to a calcium content of between 27 and 29 mg/100gm (4 ounces) of modified carrots.

2.4.2. Carrot and its medicinal properties

Ong and Chytill (1983) reported the occurrence of carotenoids in carrots and found inverse relationship with cancer occurrence.

Speek (1988) concluded that caroteniods also posses antiaging and antiulcer properties.

Deshpande (1995) said the antimutagenic, chemo preventive, photo protective and immuno enhancing properties of carrots are linked to the antioxidant properties of the carotenoids.

Hashimoto (2004) stated that carrots also possess quite a few medicinal properties, it has been reported to have diuretic and nitrogen balancing properties as well as being effective in elimination of uric acid.

Gautam (1997) reported that the production of carrot (Daucas carota L.) in India is 4.14 lakh tones in 0.22 lakh hectare with its varieties such as “Half long Nantes”, “Coreless”, “Chantaney” and “pusa kesar”.

Kotecha (1998) revealed that carrot (Daucas carota L.) is a popular root vegetable which is highly nutritious. It is a rich source of beta carotene and contains appreciable amount of thiamin and riboflavin.

Vasanthamiani (1998) reported the importance of beta carotene and vitamin A, the two important antioxidants in the diets of diabetics for reducing the incidence of complications.
Gopalan et al. (2000) reported the nutritive value of fresh carrot per 100 gm as shown:

**Table 2.4.2 Nutritive value per 100 gm of edible portion of fresh carrot:**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Value per 100 gm of edible portion of fresh carrot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>86.0 gm</td>
</tr>
<tr>
<td>Protein</td>
<td>0.9 gm</td>
</tr>
<tr>
<td>Fat</td>
<td>0.2 gm</td>
</tr>
<tr>
<td>Mineral</td>
<td>1.1 gm</td>
</tr>
<tr>
<td>Fiber</td>
<td>1.2 gm</td>
</tr>
<tr>
<td>Iron</td>
<td>1.03 gm</td>
</tr>
<tr>
<td>Carotene</td>
<td>1890 mcg</td>
</tr>
</tbody>
</table>

John (2002) concluded that carrots are nutritional heroes, they store a goldmine of nutrients and its juice is effective in treating anaemia, indigestion, rheumatism, eye problems, stones, asthma, skin disorders urinary problems and jaundice if taken along with beet root, spinach and cucumber juice.

Slattery (2002) reviewed that among vegetables; carrot is the best source of carotene, which is a precursor of vitamin A, an essential nutrient for maintaining health. Carrot contains oxycarotenoids such as lutein, which is very protective against colon cancer in men and women.

Maujunatha et al. (2003) reported that world production of carrot amounts to 18.4 million metric tonnes.

Putnam and Jane (2004) concluded that carrot in other forms are more nutritious because raw carrots have tough cellular walls, the body is able to convert less than 25 percent of their Beta carotene into vitamin A.
Tung (2005) reported that vitamin A and beta-carotene were modestly protective against ovarian cancers in smokers.

Castenmiller (2006) reported that the bioavailability of beta carotene in raw carrots was 26 percent of that of beta carotene in oil. However, in carrot juice, in which the structure of the carrot was destroyed, the bioavailability was nearly twice as high, 45 percent.

2.5 Sodium Alginate:

Clarke (2004) reported that sodium alginate is a polysaccharide of guluronic acid and mannuronic acid, which is extracted from brown seaweeds such as Macrocystis pyrifera (found off the pacific coast of North America, Australia and New Zealand) & Laminaria digitata (from Ireland, Norway, France and Scotland). It consists of a negatively charged polymer chain with ionic bonds to positively charged sodium ions (Na\(^+\)). In aqueous solution, the sodium ions dissociate from the polymer so it becomes charged. Calcium ions (Ca\(^+\)) or other doubly charged cations can bind to negative charges on two different polymer molecules. These inter molecular interactions leads to the formation of a gel. In ice cream alginates are tartrate ions to prevent. Premature gelation due to the calcium from the milk solids. The major advantages of alginate are its resistance to acid conditions particularly when heated, whereas other stabilizers would lose their functionality.