EFFECT OF INCORPORATION OF CONDENSED CHEESE WHEY AND *Bifidobacterium bifidum* IN YOGURT

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled "Effect of Incorporation of Condensed Cheese Whey and *Bifidobacterium bifidum* in Yogurt is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree diploma associateship fellowship or other similar title of any other University or Society

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CERTIFICATE

Certified that the thesis entitled "Effect of Incorporation of Condensed Cheese Whey and *Bifidobacterium bifidum* in Yogurt is a record of research work done independently by Mr Mirza Ismail Baig under my guidance and supervision and that it has not previously formed the basis for the award of any degree fellowship or associateship to him

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INTRODUCTION

Milk is a universally accepted nutritious and almost complete food versatile for its high quality proteins minerals vitamins fat and carbohydrate Lactose a reducible disaccharide is essential for better utilization of calcium and other minerals Milk fat is a concentrated source of energy

Eventhough milk contains almost all the constituents required for human system, there are certain limitations for its proper utilization

Lactose intolerance is a world wide problem in human population (Fernandes and Shahani 1989c) In the small intestine the lactose is hydrolysed to glucose and galactose in the presence of the enzyme β -galactosidase The deficiency of this enzyme in human subjects leads to abdominal pain bloating cramps loss of appetite and diarrhoea because of abnormal fermentation of lactose as it enters the colon (Hourigan 1984)

Yogurt is one of the most popular fermented milk product known for its typical aroma flavour and characteristic semi-solid consistency

Fermentation of milk by lactic acid bacteria increases its digestibility and nutritive value Lactase non-persistent individuals tolerate yogurt better than milk as measured by rise in blood glucose levels (Alm, 1982b)

The yogurt starter bacteria namely Streptococcus salivarius subsp thermophilus (S salivarius subsp thermophilus) and Lactobacillus delbrueckii subsp bulgaricus (L delbrueckii subsp bulgaricus) utilize lactose for their growth and multiplication and produce lactic acid The increased utilization of the milk sugar on consumption of yogurt is attributed to partial hydrolysis of lactose and presence of viable cells of starter culture which will continue to multiply and produce β-galactosidase during their passage in the digestive tract (Pochart et al 1989)

The therapeutic benefits attributed to yogurt consumption are reduction in serum cholesterol levels (Richardson, 1978) production of antibacterial substances and inhibition of pathogenic organisms ir the intestine (Renner 1983)

Though fortification of yogurt mix with milk powder and other source of milk solids are done primarily for improving the body and texture it also results in enhancing the nutrient contents of the product

Bifidobacteria are anaerobic lactose fermenting organisms found in the intestine of infants In breast fed infants 99 per cent of intestinal flora are composed of bifidobacteria As the age increases there is a decrease in the number of bifidobacteria and corresponding increase of other harmful bacteria (Ishibashi and Shimamura, 1993)

The greatest advantage of the presence of bifidobacteria in the intestine, either in infants or in adults is their ability to prevent the proliferation of Enterococci and Clostridia Acetic acid produced by the bifidobacteria creates an unfavourable condition for the growth of most of the pathogens The bifidobacteria has been used in conjunction with antibiotic therapy to correct the microbial imbalance in the intestine (Tojo et al , 1987)

Other beneficial effects of incorporation of bifidobacteria in regular diet include the reduction of serum cholesterol level (Homma 1988) activation and improvement in immune response system in children (Kaloud and Stogmann 1969) and anti-tumour effect (Tsuyuki et al 1991)

Starter culture of intestinal origin are reported to be more beneficial as a dietary adjunct because of better adoption in the gastro-intestinal environment (Gilliland 1979 Hoover 1993)

During the fermentation the bifidobacteria produce large quantity of acetic acid which imparts a typical insipid flavour and taste not liked by the consumers Bifidobacterial species are slow growers in milk and require some pre-formed growth promoters for their multiplication

Addition of B bifidum along with the normal yogurt culture may improve the therapeutic value of the product The acetic acid flavour produced during fermentation by bifidobacteria would also be masked by natural yogurt flavour and aroma

Whey the by-product of cheese, paneer and casein industries is a valuable source of lactose protein and ash About half of the total milk solids find their way in cheese whey Seventy five per cent of whey solids is contributed by lactose

Whey proteins are superior to casein or whole egg proteins in respect of their nutritive value The lysine and tryptophan contents of whey proteins are almost double than those of casein (Palmer 1980) They are also valuable because of their functional properties in different food formularies and baking industries

With the increased production of cheese in India and abroad disposal of whey is one of the critical problem

encountered by the dairy industry The majority of the whey is now drained off due to absence of viable economical alternative leading to tremendous loss of valuable nutrients

Whey solids and whey proteins have high Biological Oxygen Demand (BOD) and the present practice of disposal of whey without any pre-treatment is creating difficulties in the pollution control measures

The use of reverse osmosis and ultrafilteration techniques for concentration and condensation of milk and whey are very promising but these processes are yet to be adopted in commercial practice because of initial high cost and other limitation

Under the present set up of dairy industry in India the vacuum condensation of whey solids and their use in fermented milk products seems to be most practical The whey solids in concentrated form and whey protein concentrates can replace nonfat dry milk (NDM) in yogurt By this the cost of production of yogurt can be reduced and its nutritive value can be improved

Yogurt starter bacteria and bifidobacteria must remain viable during storage and during the passage through intestine The presence of viable organism in the system is

essential for producing nutritional and therapeutic effects Frozen yogurt and cultured ice cream are best known medium as carriers of these organisms in the viable state

Plain and fruit flavoured yogurt are having short shelf life During refrigerated storage the acidity continues to increase The products with higher acidity are very sour in taste and flavour High amount of acidity is also detrimental to starter culture

Frozen yogurt stored at -20°C has higher keeping quality Increased amount of milk fat sugar and presence of stabilizers and emulsifiers in frozen yogurt further improves its nutritive value

Frozen yogurt being a product having advantages of both the nutritive value of yogurt and sensory qualities of ice cream is becoming widely popular in Europe and America

Nutritional and therapeutic importance of yogurt and milk fermented with bifidobacteria are well documented Very little information however is available regarding the growth characteristics of bifidobacteria in combination with yogurt culture Bio-chemical and microbiological alterations in yogurt due to bifidobacteria are also required to be investigated

Further study on survival and stability of S salivarius subsp thermophilus L delbrueckii subsp bulgaricus and B bifidu in frozen yogurt is also necessary

The present experiment therefore is undertaken with the following objectives

1 To assess the effect of incorporation of different levels of whey solids and whey protein dispersion as a replacement for NDM on (a) growth of yogurt culture and bifidobacteria (b) biochemical and (c) sensory characteristics of yogurt and frozen yogurt

2 To study the growth performance of S salivarius subsp thermophilus L delbrueckii subsp bulgaricus and B bifidum as a mixed culture in yogurt fortified with different forms of whey solids

3 To study the effect of incorporation of B bifidum as a supplementary starter culture on biochemical and organoleptic characteristics in set and frozen yogurt

4 To study the survival and stability of the yogurt starter culture and bifidobacteria during the process of freezing and frozen storage of the products at -20°C

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2 1 Yogurt starter culture

Streptococcus thermophilus (S thermophilus) and Lactobacillus bulgaricus (L bulgaricus) have been reported as the two principal organisms used for the preparation of yogurt (Tamime and Deeth 1980)

Farrow and Collins (1984) reported a close genetic relationship between S salivarius and S thermophilus They suggested that S thermophilus should be reclassified as a subspecies of S salivarius and be redesignated as S salivarius subsp thermophilus The new nomenclature was subsequently accepted (Hardie 1986)

Hardie (1986) reported that the S salivarius subsp thermophilus was gram positive spherical to ovoid cells arranged in pairs or in long chains Lactic acid was found to be the main fermentation product from fructose glucose mannose and lactose Fermentation of sucrose by many strains of thermophilus was also reported The glucose moiety of lactose was fermented to lactic acid and galactose was either released into the medium or weakly fermented These organism was reported to produce L-lactate from glucose metabolism

The optimum growth temperature reported was 37°C with minimum 19 to 21°C and maximum 52°C

Kandler and Weiss (1986) reported a high phenotypic and genomic similarities between L delbrueckii and L bulgaricus and recommended that only L delbrueckii should be identified as a separate species and L bulgarius to be redesignated as L delbrueckii subsp bulgaricus

The morphological and biochemical characteristics of L delbrueckii subsp bulgaricus have been described by Kandler and Weiss (1986) The members of this species were gram positive rods with rounded end occurring singly or in short chains These were non-spore formers catalase negative facultative anaerobic or micro aerophilic These organism were known to produce lactate from carbohydrate metabolism Traces of acetate ethanol and carbon dioxide production were also reported The organisms were found to produce D(-) lactic acid from glucose fermentation

Kandler and Weiss (1986) also reported that optimum growth temperature for L delbrueckii subsp bulgaricus was 45°C Pantothenic acid and niacin were reported to be essential growth factor For some strains riboflavin folic acid vitamin B_{12} and thymidine were also essential

2 1 1 Symbiotic relationship between yogurt starter culture

Pette and Lolkema (1950a) demonstrated the symbiosis between S thermophilus and L bulgaricus They observed that the rate of acid production was greater when the two yogurt bacteria were grown together in milk than grown separately They also found that the number of cocci were much higher in mixed culture than in a single culture under a similar conditions of incubation Tamime (1977) however reported that population of both rods and cocci were much higher when grown in mixed culture than in single culture under similar conditions

Bautista et al (1966) identified the compounds responsible for the symbiotic growth of S thermophilus and L bulgarius They found that histidine and glycine were produced during fermentation by L bulgarius and the fortification of histidine and glycine resulted in higher acid production in milk by the single culture of thermophilus They concluded that the histidine and glycine produced by the bulgaricus was utilized by the thermophilus for their growth during the fermentation of milk by the mixed culture

Galesloot et al (1968) found that under anaerobic conditions the S thermophilus produced stimulatory factors which favoured the growth of L bulgaricus Veringa et al

(1968) found that the stimulatory factor produced by thermophilus was formic acid

Among the two yogurt starter bacteria L bulgaricus was better able to hydrolyze casein whereas S thermophilus had significant peptidase activity for hydrolysing the products of initial casein breakdown Consequently the proteolytic activities of the two starter culture bacteria complement each other and contribute to cooperative growth (Moon and Reinbold 1976)

Tamime and Robinson (1985) reported that the pattern of proteolysis by the yogurt culture would also help the two organisms for their symbiotic growth The L bulgaricus possessed more proteinases activity and hydrolysed casein to form polypeptides The peptidases synthesized by S thermophilus was responsible for breaking of the peptides and the liberation of free amino acids

Driessen et al (1982) designed an experiment to study the requirement of carbon dioxide (CO_2) for optimum growth of L bulgaricus Amount of carbon dioxide produced by S thermophilus in mixed culture and effect of heat treatment on carbon dioxide level in milk was also studied They observed that for the optimum growth of L bulgaricus more than 31 mg of CO_2 per kg of milk was essential The initial

carbon dioxide level in yogurt mix was 60 mg in one kg During heat treatment of 85°C for 5 minutes the concentration of the gas was reduced to less than 6 to 10 mg per kg After the inoculation of the mix with the two yogurt culture it was found that S thermophilus produced 50 mg of carbon dioxide per kg of the mix in first 45 minutes of incubation The production of the carbon dioxide continued throughout the incubation period

2 1 2 Polymer producing yogurt culture

Cho Ah Ying et al (1990) prepared yogurt from the combination of two types of yogurt culture viz RR and SW, in a ratio of 20 80 Both the culture were having bulgaricus and thermophilus in 1 1 ratio Thermophilus in RR culture however, had an ability to produce polysaccharides It was found that by the use of polymer producing cultures yogurt with improved consistency and texture ould be prepared

Marshall (1993) suggested the use of polymer producing variants of the starter cultures to overcome the textural problem in yogurt Both S salivarius subsp thermophilus and L delbrueckii subsp bulgaricus were able to produce polymers containing glucose galactose rhamnose and uronic acid residues These polymers were respon>ible for improving the textural characteristics of the produc

Rodrate et al (1993) studied the rate of syneresis apparent viscosity and sensory characteristics of yogurt prepared from polymer producing (ropy) strains of yogurt culture The products were prepared from the mix having 12 14 5 and 17 per cent of total solid They found that rate of synersis was very low in yogurt from the ropy strains even at 12 per cent of total solids They suggested that a good quality yogurt with reduced cost could be prepared by using polymer producing culture with only 12 to 14 5 per cent total solids

2 1 3 Generation time of yogurt culture bacteria

Mitchell and Sandine (1986) reported that the generation time of S thermophilus varied from 51 7 minutes to 109 3 minutes and that of L bulgaricus was 40 2 to 77 9 minutes The generation time for each species varied from strains to strains

2.2 Bifidobacteria

2 2 1 Morphological and physiological characteristics

Scardovi (1986) has described the morphological and physiological properties of different species of bifidobacteria B bifidum were reported to be found in the intestine of breast fed infants These were gram positive

rods of different shape The organism could be of different forms such as short regular thin cells with pointed ends coccoidal regular cells long cells with slight bends or with protuberances They might also found as club shaped or with spatulated extremities and also in star like aggregates or disposed in V or palisade arrangement The colonies of bifidobacteria species were generally smooth convex entire edge cream to white glistening and were of soft consistency These organisms were found to be non-acid fast non-spore formers non-motile Cells often stained irregularly with methylene blue These were generally anaerobic however some species could tolerate oxygen in the presence of carbon dioxide

Scardovi (1986) reported that the optimum growth temperature for majority of bifidobacteria species was 37-41°C However they can also grow at 25-28°C and could tolerate the temperature upto 45°C Optimum pH for initial growth was 6 5 7 0 Bifidobacteria were found to produce acetic acid and lactic acid in the molar ratio of 3 2 Carbon dioxide was not produced B bifidum could ferment lactose galactose fructose and sucrose Riboflavin nicotinic acid folic acid and p amino benzoic acid were reported to be essential for their growth

A water soluble bifidus growth stimulator (BGS) was isolated from the cells of Propionibacterium freudenreichii 7025 by Kaneko et al (1994) The BGS was found to be present in cell free extract as well as within the cells of P freudenreichii It was also reported that several intestinal bacteria such as Bacteriodes Enterobactor and Entrococcus stimulate the growth of bifidobacteria

The growth of B adolescentis 6003 was found to be inhibited in the mixed culture with Clostridium perfringens 7028 (Kaneko et al 1994) However they found that the growth of B adolescentis 6003 was greatly enhanced even in presence of C perfringens in media with short chain fatty acids and BGS produced by P freudenreichii 7025

2 2 2 Carbohydrate metabolism in bifidobacteria

The carbohydrate metabolism in the 17 strains of B bifidum was studied by De-Varies and Stouthammer (1967 1968) The Fructose-6 phosphate phosphoketelase was found be an important enzyme required for the glucose metabolism in those strains Aldolase and glucose 6-phosphate dehydrogenase the enzymes reported to be essential for carbohydrate metabolism in homofermentative lactic acid bacteria were not detected in bifidobacteria In their experiment De Varies and Stouthammer (1968) found that the

acetate L(+) lactate ethyl alcohol and formate were the major end products from glucose glalactose lactose mannitol and xylose fermentation in different strains of bifidobacteria

Frank and Marth (1988) pointed out that glucose fermentation in bifidobacteria involved two phosphoketolase enzymes one for fructose 6 P and other sepcific for xylulose 5 P As such the pathway oi glucose metabolism in bifidobacteria was found to produce 5 moles of ATP for every two moles of glucose metabolised

2 2 3 Cultured dairy products with bifidobacteria

Schular Malyoth et al (1968) reported that the bifidobacteria were slow growing and non competitive in the presence of other lactic acid bacteria These organism required free amino acids for the initiation of their growth in milk He found that with higher inoculum sufficient number of bifidobacteria could be propagated in the milk He advocated the following scheme for the preparation of a cultured milk product containing B bifidum S thermophilus and Lactobacillus acidophilus Single culture of these organisms were incubated for 7 4 and 24 hours respectively at 12°C A bulk starter containing three organisms in 1 1 1 was prepared and incubated further for four hours at 42°C The

milk was then inoculated with the bulk starter and incubated again for three hours at similar temperature B bifidum population in the product was 10^6-10^8 colony forming unit (cfu) per ml

A fermented milk with B bifidum was prepared by Marshall et al (1982) using ultrafiltered skim milk fortified with ultrafiltered cheese whey and threonine The total solids content in the mix were adjusted to 15 per cent The mix contained 7 3 per cent proteins 4 95 per cent lactose 1 3 per cent fat and 1 45 per cent ash The product obtained after 24 hours of incubation at 37°C had a good aroma and consistency similar to that of a conventional yogurt The fresh product was having $3x10^9$ cfu of B bifidum per ml and after 21 days of storage at 4°C the viable count was $5\ 2x10^7$ cfu per ml

Anand et al (1984) suggested the addition of one per cent dextrose and 0 1 per cent yeast extract in the milk whereas Collins and Hall (1984) advocated the supplementation of cystine and pyruvic acid for satisfactory growth of B bifidum

Goh et al (1986) found tha B bifidum ATCC 11863 population in reconstituted skim milk having nine per cent total solids was $8\ 2 \times 10^8$ cfu per ml Fortification of the

skim milk with L cystine (0 05 per cent) and yeast extract (0 2 per cent) and increasing the inoculum rate from 2 to 5 per cent increased the B bifidum count to 6 7 x 10^9 cfu per ml

Zbikowski and Zikjka (1986) reported that enrichment of reconsituted skim milk with five per cent carrot juice improved the acidity production by B bifidum They had developed a humanized infant formula with milk solids ultrafiltered whey protein concentrate, soyabean oil, carrot juice lactose and sucrose It was found that the acid production by B bifidum in the humanized infant formula was higher (17 8° SH) than in commercial infant formulae (9 0 16 7° SH)

Tamime and Robinson (1988) suggested the use of bulk starter rather than direct to vat inoculation as a means for reducing production time and ensuring higher numbers of viable cells of bifidobacteria in the end product

Laroia and Martin (1990) reported that B bifidum produced L(+) lactic acid whereas L acidophilus formed DL isomers and D(-) by L bulgaricus It was also reported that L(+) lactic acid was completely metabolised by infants in the synthesis of glucose and glycogen ind was also used in respiratory process The lactic acid with D(-) isomer

however was metabolized at a slower rate and caused metabolic acidosis For these reasons Laroia and Martin (1990) argued that the bifidobacteria had more beneficial role than bulgaricus as a dietary adjunct

Puhan (1990) however found that human muscles produced D( ) lactate in small quantity and its production was considered as a normal physiological phenomenon The accumulation of D(-) lactate had not produced any harmful effect

Human milk was reported to be a better substrate for growth and multiplication of bifidobacteria because of the presents of the bifidus factor (Laroia and Martin 1990)

Hoover (1993) however argued that no such bifidus factor was present and better growth of bifidobacteria in human milk was due to lesser protein content and diminished buffering capacity

Desjardins et al (1990) studied the fermentation characteristics and enzyme profiles of different strains of bifidobacteria of infant and adult origin They found that B bifidum 15696 and 29521 and B longum isolated from infant intestine grew faster in milk than B adolescentis which was of adult origin Their findings suggested a close relationship between environmental and nutritional conditions

in the intestine and growth performance of different strains of bifidobacteria in milk All the strains of bifidobacteria showed high α and β galactosidase activity They also exhibited higher glycosidase activity than other intestinal flora

According to Desjardins et al (1990) α-galactosidase an enzyme responsible for hydrolysis of specific sugars such as α D galactosyl oligosaccharides was found to be important for the selective proliferation of bifidobacteria in human intestinal tract

They also found that N acetyl D glucosaminidase activity was present in all bifidobacteria of infant origin and was absent in that of adult origin The highest activity the enzyme was found for strain B bifidum Var pennsylvanicus (ATCC 11863) a slow growing strain in milk

Modler et al (1990) found that incorporation of cell biomass of B longum B brevi and B infantis into the ice cream mix and subsequent batch freezing had a only slight effect on the survival of these organisms Approximately 90 per cent of bifidobacterial cells survived during freezing and storage for 70 days at 17°C

In vivo and *in vitro* studies on survival of bifidobacteria during gastric transit and in acidic environment was carried out by Berrada *et al* (1991) Their findings revealed that resistance to gastric environment varied between different strains of bifidobacteria The passage of the bifidus milk in stomach was monitored by non-absorbable radio active isotopes It was found that 50 80 per cent of the product passed into the intestine within 45 90 minutes The initial bifidobacteria population in the product was $6x10^7$ per gram During gastric transit the population was decreased by less than two logarithmic unit

Misra and Kuila (1991 1992) used 10 per cent inoculum of *B bifidum* for preparation of cultured bifidus milk from cow and bufffalo milk They found that after 18 hours of incubation at 37°C the developed acidity was 0 78-0 86 per cent and total viable count was $4x10^9$ cfu per ml

Samona and Robinson (1991) used sterile skim milk with 12 per cent total solids without any supplementation for culture maintenance of *B bifidum* They used different selective and non-selective media for enumeration of bifidobacteria in reconstituted skim milk The average count were 13 $5x10^8$ cfu per ml

Rogers (1991) enlisted the following criteria for selection of bifidobacteria strains of right type (a) should produce acetic and lactic acid in the ratio of 3 2 and attain a pH of 4 7 in milk within four to eight hours at 38°C by using 10 per cent inoculum (b) should possess fructose 6 phosphate phospho-ketolase enzyme (c) should be negative for the following tests catalase urease nitrate reductase formation of indole liquification of getatin and gas formation from glucose (d) should possess sufficient acid resistance to survive storage in products like yogurt (e) should be tolerant to oxygen resistant to bile and common antibiotics used in human medicine

Micro encapsulation of the cells of bifidobacteria by gelatin vegetable gum butter fat or by modified starch was recommended for improving the survival rate of the organisms in acidic condition (Rogers 1991)

Murti et al (1992) compared the growth of bifidobacteria in soya extract in cow milk supplemented with yeast extract and in unsupplemented cow milk The organism did not grow in unsupplemented milk Growth was more rapid in supplemented milk than in soya extract The population of bifidobacteria in supplemented milk was $1x10^{10}$ per ml and in soya extract the count of $2\ 5x10^{9}$ per ml was reported

Ishibashi and Shimamura (1993) reported that the fermented milk products containing bifidobacteria and L acidophilus (like cultura AB) were having the highest demand because of their nutritional and therapeutic values In terms of consistency and flavour these products resembled natural yogurt

It was suggested that the cultivation of S salivarius sub sp thermophilus with bifidobacteria would be beneficial The release of carbon dioxide by the thermophilus would be stimulatory for the growth of bifidobacteria (Ishibashi and Shimamura 1993)

Ventling and Mistry (1993) studied the growth of B bifidum and B longum in ultrafiltered milk They reported an increase in buffering capacity of ultrafiltered milk due to concentration of proteins and salt The acid production by both the bifidobacterial species was lower in ultrafiltered milk however the bacterial count showed no difference in ultrafiltered and normal skim milk It was also indicated that the generation time for B longum was shorter (20 3 30 3 minutes) than B bifidum (66 2 134 4 minutes)

2 3 Importance of whey solids and their uses

2 3.1 Composition of cheese whey

Cheese whey was reported to contain seven per cent total solids which was about half of the total solids of whole milk The major component of the whey was found to be lactose (4 9 per cent) The content of protein fat and ash were 0 9 0 3 and 0 6 respectively (Nutting 1970)

Palmer (1980) analysed whey proteins and casein for their amino acid profile The lysine content of whey proteins was 10 9 per cent as against 7 6 per cent in the casein The percentage of cystine in whey proteins and casein was 3 15 and 0 4 respectively The total essential amino acids in whey was higher (62 55 per cent) than in casein (49 45 per cent)

The principal whey proteins were β-lactoglobulin α lactalbumin bovine serum albumin and different immuno globulins β lactoglobulin was found to be the major whey protein representing 50 per cent of total whey nitrogen (Whitney 1988)

2 3 2 Functional properties of whey proteins

Solubility of the proteins was considered as an important functional property and was found to be a pre requisite for most other functionalities in food products

(Mulvihill and Fox 1989) They found that rennet casein acid casein and casein coprecipitate were insoluble in water Undenatured whey proteins however were soluble in wide pH range and could be used as a replacement for chemical stabilizers in food products

Devit (1989) suggested that whey proteins in concentrate form could be incorporated in a wide range of food products In undenatured form they were soluble and could exert gelling foaming emulsifying and water binding properties in food and dairy products

Pearce and Marshall (1991) reported that β-lactoglobulin and α-lactalbumin possessed foaming and emulsifying activities equal to egg white proteins and should be considered for the replacement for xpensive agents They also found that denatured whey proteins could be used to control viscosity in food

2 3 3 Method of concentration of whey solids

Vacuum concentration of whey at temperatures less than 60°C to increase its keeping quality and to reduce the volume was in practice since middle of this century (Nutting 1970)

To avoid protein denaturation Marshall (1982) recommended that the temperature during vacuum concentration

should be less than 70°C By this procedure whey could be concentrated to 50 60 per cent total solids

Concentration of whey solids by membrane processing techniques like reverse osmosis and ultrafilteration were of recent origin Whey could be concentrated to 25 per cent total solids without affecting nutritional and functional properties of whey proteins (Marshall 1982 Morr, 1989 Patel et al 1991 and Nanjundaswamy 1992) One of the advantage claimed for membrane processing besides low energy requirement was that it did not cause any chemical changes in different whey components

2.3 4 Preparation of whey protein concentrate

Marshall (1982) reported that heat denaturation and acid precipitation for recovering of whey proteins was found to be one of the oldest method This method retained the nutritive value of proteins however the functional properties were lost by heat denaturation

Morr (1989) found that whey protein concentrate prepared by ultrafiltration of cheddar cheese whey contained 78 per cent water 13 4 per cent proteins and 4 1 per cent lactose There was no denaturation and all the functional properties of the proteins remained unaffected

Vishweshwariah and Ramanathan (1991) pointed out that the process of reverse osmosis and ultrafilteration were quite expensive for recovering whey proteins They developed a method of preparation of whey protein concentrate (WPC) Fresh cheese whey was vacuum concentrated to 60 per cent total solids and blended with coagulated whey proteins in a ratio of 1 1 5 by weight The latter component was obtained by coagulating another batch of cheese whey with two per cent citric acid at 80°C After cooling to 35°C the whey was centrifuged and sediment of whey protein was collected The mixture of the two product was homogenized by a single stage homogenizer and dried in a vacuum shelf drier at the temperature of 65°C with vacuum of 28 inches of mercury The dried whey protein concentrate was having 36 per cent protein 51 per cent lactose 5 2 per cent fat 4 8 per cent ash and three per cent water

2 3 5 Incorporation of whey solids in dairy products

Mathur and Shahani (1979) pointed out that whey solids in dried or condensed form could be incorporated in cheese milk infant food formulae special dietetic food beverages bakery products dried culture media and in ice cream mix In these products whey solids could be used in the range of three to 97 per cent Whey solids besides contributing the nutrient rich ingredients were found to help in flavour and texture

improvement Lactose the major component of whey solids acted as a carrier of flavour in food and dairy products They further indicated that the largest single use of whey solids in dairy products was in ice-cream to replace non-fat dry milk (NDM) United States Federal regulation permit 25 per cent replacement of NDM by whey solids

Broome et al (1982) used ultrafiltered Cheddar cheese whey to replace NDM in yogurt The content of protein lactose and ash after ultrafilteration of the whey were 5 8 4 8 and 0 7 per cent respectively The total solids were 12 8 per cent The whey was used to replace 12 20 25 and 30 per cent of the NDM in the yogurt They ound that replacement of NDM upto 25 per cent did not affect the taste and textural properties of yogurt

A fermented milk product having typical yogurt flavour with smooth consistency was prepared with the combination of ultrafiltered skim milk and ultrafiltered cheddar cheese whey, in 3 1 proportion (Marshall et al 1982) Starter culture of Lactobacillus acidophilus was used for fermentation At a pH 4 2 the viable acidophilus count was $1x10^8$ cfu per ml

Guirguis et al (1984) studied the viscosity and syneresis in yogurt fortified with spray dried whey protein concentrate (WPC) They found that incorporation of WPC to

replace 50 per cent of NDM had resulted in improved viscosity and reduced syneresis

Abd-El-Salam et al (1991) prepared yogurt from whole buffalo milk and compared it with yogurt prepared from the milk fortified with ultrafiltered whey protein concentrate They found that fortification of WPC at a level of 20 per cent of the total mix improved the texture and mouth feel and reduced the synersis in yogurt

Opdahl and Baer (1991) conducted a consumer s acceptance survey of frozen yogurt The product was prepared by replacing 100 per cent NDM with spray dried whey protein concentrate The protein and carbohydrate content of whey protein concentrate was 34 1 and 51 1 per cent respectively They found that 87 8 per cent consumers had accepted this product

2 3 6 Use of whey solids in starter medium

Mathur and Shahani (1979) reported that whey based media with seven per cent total solids supported better growth for Streptococcus lactis S thermophilus L bulgaricus and L acidophilus than media prepared from reconstituted skim milk with ten per cent total solids

Modler and Villa-Garcia (1993) developed an inexpensive whey based medium for large scale production of bifidobacteria The medium contained 11 per cent whey solids 0 05 per cent cystine and 0 23 per cent yeast extract Bifidobacteria count in the order of 10^{10} were obtained in the culture medium

2 4 Preparation of the basic mix for yogurt

Milk and other raw materials used for the manufacture of yogurt should be of very good quality Any off flavours in raw milk could be carried over in the finished dairy product (Norling 1979 and Chambers 1979)

2 4 1 Standardisation for fat and solid not fat

The consistency and aroma of the yogurt was depended on the level of the total solids in the product (Tamime and Deeth 1980) They recommended that the total solids in the best quality yogurt should be 15-16 per cent

The fat percentage in yogurt varied from 0 5 per cent to 3 8 per cent depending on the legal standards in different countries (Tamime and Deeth 1980)

The desired level of total solid in the yogurt mix could be achieved by the partial evaporation of the milk

(Norling 1979) fortification of skim milk powder (Tamime and Robinson 1988) or by the fortification with condensed whey and whey protein concentrate (Abd El-Salam 1991 and Pearce and Marshall 1991)

2 4 2 Concentration/standardisation of total solids in yogurt mix by membrane processing

Jepsen (1977 1979) reported that whole milk concentrated by ultrafilteration to 18-20 per cent total solids produced a smooth creamy yogurt with typical acid flavour

Abrahamsen and Holman (1980) found that yogurt prepared from ultrafiltered milk gave a rirm coagulum and high viscosity than that fortified with skim milk powder

Dixon (1985) reported that yogurt prepared from skim milk concentrated by reverse osmosis (RO) was more viscous with less synersis and was having typical flavour and texture He suggested that the increase in viscosity of RO concentrated yogurt would allow the manufacturer to reduce solid not fat (SNF) and stabilizers content of the product

2 4 3 Sweetening agents in yogurt

Sweetening agent usually sugar was added in yogurt

to tone down the acidic taste and flavour (Tamime and Robinson 1985)

A reduction in the rate of acid development by S thermophilus and L bulgaricus was noted as the sugar content in the yogurt increased from six to 12 per cent (Tramer 1973) It was also found that S thermophilus was more tolerant to high sugar concentration than L bulgaricus

Tamime and Robinson (1985) reported that besides sugar invert sugar, fructose glucose sorbitol and saccharin could be used in yogurt depending on availability, cost legal and nutritional aspects

2 4 4 Homogenization

Homogenization is the proce s by which the fat globules in milk or cream are broken down into small size by mechanical force Due to reduction in globule diameter there will be an increase in the surface area of fat globule by four to six times The increase in sui face area was to be responsible for increase in viscosity ind whiter appearance of the product The small sized globules would have less tendency for clumping and rising to the surface of the product (Tamime and Robinson 1988)

2.4.5 Heat treatment of milk

Labropoulos et al (1984) found that the heat treatment of milk with different time temperature combinations influenced the body and texture of yogurt They compared the effect of three types of heating processes viz ultra-heat treatment (UHT) at 149°C for 3 3 seconds vat pasteurization at 63°C for 30 minutes and conventional system with 82°C for 30 minutes It was found that heating of the yogurt mix for 82°C for 30 minutes resulted in a product with higher gel firmness higher curd tension more viscosity and lesser relative fluidity and spreadability than that of UHT and vat pasteurization method They had attributed the increased water holding capacity of the milk proteins as a reason for the enhanced viscosity of the product

Tamime and Robinson (1985) suggested that the heat treatment of yogurt mix was essential for destruction of pathogens and other undesirable organisms

Parnell-Clunies et al (1986) reported that yogurt firmness and viscosity were increased with the vat process of heat treatment (85°C for 10-40 minutes) of the mix as compared to high temperature short time (HTST 98°C for 0 5-1 87 minutes) and UHT (140°C for 2-8 seconds) Water

holding capacity of the coagulum however was highest with HTST process followed by UHT and vat treatments

Frank and Marth (1988) reported that the different heat treatments either inhibit or stimulate the growth of starter culture Heating the milk at 62° 72°C for 30 minutes or 90°C for 60-180 minutes or autoclaving at 120°C for 15-30 minutes stimulated the growth of the starter culture However heating milk at 72°C for 45 minutes, or 82°C for 10-45 minutes or at 90°C for 1-45 minutes was inhibitory for starter growth The stimulatory effect of heat treatments was attributed to expulsion of oxygen with subsequent lowering of the oxidation-reduction potential destruction of inhibitors partial protein hydrolysis and serum protein denaturation

2 4 6 Incubation temperature

Chambers (1979) reported that careful selection of incubation temperature was essential for culture balance acidity texture and flavour of the yogurt Rapid acid development contributed to wheying off defect and poor gel development

Tamime and Robinson (1985) advocated that both short set (40 45°C for 2 5 3 5 hours) or long set (30°C for 18 hours) methods of incubation of yogurt could be practiced

under industrial conditions depending on the type and method of subsequent cooling

Cho-Ah-Ying et al (1990) found that the temperature of incubation (38°C Vs 43°C) did not significantly affected acidity and the viscosity of set and stirred yogurt However the yogurt prepared at 38°C scored higher in sensory evaluation than at 43°C

2 5 Frozen yogurt

Gilliland (1979) emphasized that the culture bacteria must be in a viable state during storage and at the time of consumption of the product so that they could exert their beneficial effect in the intestine

Fernandes and Shahani (1989c) stressed on the presence of viable bacterial cell in yogurt to have its beneficial effect to lactose intolerant individuals than a mere reduction in lactose content in fermented milk products Their studies showed that yogurt containing live bacterial cells was better tolerated by lactose intolerant subjects than pasteurized yogurt

Salji and Ismail (1983) reported that the acidity of plain yogurt was progressively increased during refrigerated

storage They also found that the product with high acidity was not acceptable to the consumers

The works of Modler et al (1990) and Mashayekh and Brown (1992) had indicated that β galactosidase activity and colony counts of S salivarius subsp thermophilus and L delbrueckii subsp bulgaricus were progressively decreased during 31 days of storage at 4°C However, they found that there was only a slight reduction of population of thermophilus bulgaricus and bifidobacteria in frozen yogurt and cultured ice cream stored at -17°C to 29°C

Freezing of the plain yogurt without standardisation of fat and total solids resulted in the formation of large ice crystals (Mashayekh and Brown 1992) The presence of large ice crystals caused the textural defects in frozen yogurt and was unacceptable to consumers (Donhowe et al , 1991) The large ice crystals were also detrimental to the survival of starter organism (Sheu et al , 1993) Mashayekh and Brown (1992) however found that fortification of fat sugar stabilizers and emulsifiers reduced the ice crystal size and imparted a soft smooth texture to the frozen yogurt

2 5 1 Composition and method of preparation

Knupp (1979) recommended the following composition for both soft serve and hard frozen types of yogurt Butter fat

one to two per cent NDM 9 10 per cent cane sugar 11 5-12 5 per cent corn syrup solids 8 8 5 per cent fruits 15 per cent and stabilizers 0 25-0 45 per cent

To avoid inhibition of culture activity during incubation due to excessive sugar and total solids Knupp (1979) prepared the frozen yogurt in two stages Plain yogurt with normal composition having 16 per cent total solid was prepared in first stage Mixture of sugar stabilizers and emulsifiers were heat treated and after cooling mixed with fermented yogurt

Kosikowski (1981) analysed 24 brands of commercial frozen yogurt for their proximate composition He found that content of fat total protein carbohydrate ash and total solids were 0 8 2 5 1 7-4 5 17 5-34 0 0 7 1 2 23 6 38 9 per cent respectively He further reported that titratable acidity ranged from 0 31 1 35 per cent and pH 4 0-6 5 Some of the frozen yogurt samples were having very less acidity and did not have typical yogurt flavour

Opdahl and Baer (1991) used whey protein concentrate to replace NDM in frozen yogurt The composition of the product was as indicated below The percentage of milk fat was six whey protein concentrate 10 5 sucrose 11, corn syrup solids three per cent stabilizers and emulsifiers 0 3 and

total solids 30 8 The titratable acidity in the product was 0 24 0 46 per cent

Mashayekh and Brown (1992) found that higher percentage of total solids (i e 40 32 per cent) in the ice cream mix did not affect the growth and acid production by the thermophilus and bulgaricus They used yogurt starter culture for the preparation of cultured ice cream The composition of the mix was as under Fat 12 per cent SNF 11 per cent sugar 12 5 per cent corn syrup solids 4 5 per cent stabilizers and emulsifiers 0 32 per cent The mix was inoculated with one per cent yogurt starter culture After four to five hours of incubation at 43°C the desired pH i e 4 9 was obtained The product was cooled in an ice-bath with agitation to below 10°C and aged overnight at 4°C The mix was frozen in batch ice cream freezer and hardened to -29°C They claimed that this method gave a product with smooth consistency and good flavour

Similar composition for the ice cream mix and similar method was adopted for preparation of probiotic ice cream by Hekmat and McMahon (1992) Four per cent inoculum each of B bifidum and L acidophilus was used for fermentation The desired pH of 4 9 was attained after five hours of incubation at 42°C

2 6 Starter count in yogurt

2 6 1 Viable counts of S salivarius subsp. thermophilus and L. delbrueckii subsp bulgaricus in yogurt

In order to ensure a desirable flavour aroma and firm consistency Kroger (1976) has suggested a ratio of 1 1 for thermophilus and bulgaricus in the final product The balance of these organisms can be maintained by the control of incubation temperature selection of proper strains of starter culture and the rate of inoculum of the individual organism (Kroger 1976 and Chambers 1979)

Kroger (1976) found that the total plate count in yogurt increased from initial 26 millions to 700 million per ml after 10 days of storage at 7°C

Chambers (1979) analysed 152 commercial samples of yogurt for thermophilus and bulgaricus count He found that only 15 per cent of the samples were having 1 1 ratio L bulgaricus were dominant in 44 per cent of the samples and S thermophilus were more in 41 per cent of yogurt samples

Broome et al (1982) reported that the fortification of whey protein concentrate in yogurt stimulated the growth of S thermophilus however the same effect was not evident for L helveticus

Labropoulos et al (1982) found that by the inoculation of the mix with 1 % per cent each of S thermophilus and L bulgaricus a proper ratio of 1 1 of viable cells could be maintained

Abou Donia et al (1984) evaluated the 20 commercial samples of yogurt prepared from buffalo milk (Zabady) for total bacterial count He found great variation in total count which ranged from 1 35 x 10^6 to 1 56 x 10^7 cfu per ml

In their experiment to test the compatibility of different strains of thermophilus and bulgaricus at different incubation temperature in milk Mitchell and Sandine (1986) found that the temperature of incubation and optimum growth temperature for individual strains did not have any influence on their ratio He also found that fortification of mix with milk solids resulted in increased number of viable cells

Salji et al (1987) studied the shelf life and total lactic count of commercial yogurt samples They found that there were no increase in coliform yeast and mould count during storage at 7°C for 5 days The total lactic count ranged from 3 5 x 10^7 to 9 2 x 10^8 at the time of the sale of the product

Prasad (1990) reported that storing raw milk pasteurized milk and Lactoperoxidase activated milk for 24 to

72 hours at refrigerated temperature prior to preparation of yogurt did not affect the thermophilus and bulgaricus count in the product The average count for thermophilus was 2 33 x 10^8 cfu per ml and that of bulgaricus was 2 59 x 10^8

The findings of Gassem and Frank (1991) revealed that the incorporation of bacterial protease or purified plasmin in yogurt mix prior to fermentation stimulated the growth and acid production of yogurt starter culture The time required to attain iso-electric point during incubation was also significantly lesser in enzyme treated mix than the control However the thermophilus and bulgaricus count in enzyme treated and control samples of yogurt did not differ significantly

Hamzawi and Kamaly (1992) made the stirred yogurt from buffalo milk and enriched with 10-30 per cent cooked wheat grains Plain yogurt was prepared using two per cent yogurt starter culture After complete coagulation cooked wheat grains were added at the rate of 10 20 and 30 per cent The products were stored for seven days at refrigerated temperature They found that the thermophilus and bulgaricus count were higher in yogurt enriched with cooked wheat grain than in control After seven days of storage, the total lactic counts in control sample were log 8 08 and in enriched samples were log 8 32 cfu per ml

Kim et al (1992) conducted an experiment on the effect of mechanical shaking on viable count of thermophilus and bulgaricus in yogurt Samples of the product were shaken at 100 and 200 revolution per minute immediately after the coagulation The objective of thei investigation was to determine the extent of damage to th viable bacterial cells during transport and delivery of th stirred yogurt They found that mechanical shaking did not have any adverse effect on survival of yogurt bacteria

Kim et al (1992) also studied the effect of storage of the yogurt at 10 and 20°C on the starter bacterial cells Samples of the products were stored for 15 days It was found that viable cells of thermophilus remained constant at more than 1×10^9 cfu per ml during the 15 days storage period at 10°C However the count rapidly decreased to $1\ 2 \times 10^6$ cfu per ml at 20°C The bulgaricus count was also decreased to $2\ 6 \times 10^6$ cfu per ml at 20°C after six days of storage and continued to decrease further throughout the storage period

2 6 2 Viable count of bifidobacteria in association with yogurt starter culture

Robinson (1990) studied the survival of B bifidum in yogurt Fermented product was prepared using 1 5 per cent inoculum of B bifidum and 0 5 per cent yogurt culture The

mix was incubated to pH 4 0 to 4 5 Fruit syrup was added after fermentation and stored at refrigerated temperature for 21 days The B bifidum count after 21 days of storage was more than 1 x 10^9 cfu per gram of the sample

Martin and Chou (1992) studied the viability of B bifidum B longum B infantis B adolescentis and B breve in low pH (pH 4 2) and in high pH (pH 5 6) yogurt They found that the population of bifidobacteria declined rapidly in yogurt having higher acidity It was also reported that survival rate of bifidobacteria in yogurt was strain and species dependent

Murti et al (1992) compared the S salivarius subsp thermophilus L delbrueckii subsp bulgaricus and bifidobacteria count in yogurt and in product prepared from soya milk They found that the thermophilus developed better in the presence of bifidobacteria however, the bulgaricus growth was inhibited The bifidobacteria remained stable in both yogurt and soya-yogurt at 1 x 10^7 per ml They also indicated that soya milk was better substrate for thermophilus and bulgaricus than milk

2.7 Viable count of thermophilus, bulgaricus and bifidobacterial in frozen yogurt

Kosikowski (1981) and Meyer (1989) found great

variations in the lactic acid bacterial count in commercial frozen yogurt The viable bacterial cells varied between 3 x 10^5 to 6 4 x 10^8 in 23 commercial samples of frozen yogurt The ratio of thermophilus to bulgaricus was 1 1 in majority of samples Some samples with 70 per cent thermophilus and 30 per cent bulgaricus were also encountered

Modler et al (1990) incorporated viable cell biomass of B adolescentis B longum and B infantis into the ice-cream mix before freezing The bifidogenic factors in the form of Jerusalem antichoke flour and fructo oligo-saccharides were also incorporated into the mix It was pointed out that these two agents were non-nutritive sweeteners and not metabolised by human digestive system However these could be utilized by bifidobacteria in the human colon as a source of energy In their experiment Modler et al (1990) found that addition of the bifidogenic factors did not have any adverse effect on bifidobacteria counts in the ice-cream However ten per cent decrease in the starter count was observed during the blending of the organism with the mix and freezing There were no change in bifidobacteria population during storage of the ice-cream at -17 C for 70 days After the end of the storage period the average bifidobacteria count was log 7 0 cfu per ml

Mashayekh and Brown (1992) prepared a cultured ice-cream by fermenting the mix with yogurt starter culture Ice-cream mix was first made with 12 per cent fat 11 per cent SNF 12 5 per cent sugar 4 5 per cent corn syrup solid and 0 32 per cent stabilizer The mix was inoculated with one per cent yogurt culture After fermentation the mix was frozen with batch freezer and hardened at 29°C They found that during freezing process there was only a slight decrease in number of bacteria Storage of product at 29°C for one month did not affected the count of thermophilus and bulgaricus significantly

Probiotic ice cream was made by fermenting a standard ice cream mix with L acidophilus and B bifidum (Hekmat and McMahon 1992) The mix was frozen in a batch freezer and stored at 29°C After freezing and hardening bacterial count were 1 5 x 10^8 cfu per ml for L acidophilus and 2 5 x 10^8 cfu per ml for B bifidum Seventeen weeks after freezing these counts had decreased to 4 x 10^6 and 1 x 10^7 cfu per ml respectively

In an experiment to study the viability of L acidophilus and B bifidum in soft serve frozen yogurt Holcomb et al (1991) found that both organism survived freezing and continued to grow during storage at -5°C

Sheu et al (1993) developed a method of micro-entrapment of bacterial cells in the beads of calcium alginate to protect the cells during freezing and hardening They reported that L delbrueckii subsp bulgaricus survived still freezing (without agitation) in ice milk mix much better than in distilled water Entrapment protected the bulgaricus cells in batch and in continuously frozen ice milk In continuous frozen milk the percentage of survival was 90 per cent for entrapped cells and 40 per cent for unprotected cells Count of the bacterial population in soft ice milk prior to hardening indicated that most of the death occurred in freezer due to continuous agitation rather than during hardening process They concluded that alginate matrix not only protected the entrapped bacterial cells during frozen storage but also from mechanical damage during freezing

2 8 Proteolysis in yogurt

Proteolysis of casein by starter culture was found to be important for optimum flavour and textural characteristics of yogurt L delbrueckii subsp bulgaricus could hydrolyse casein whereas S salivarius subsp thermophilus showed significant peptidase activity for breaking down the products of casein hydrolysis The proteolytic activities of the two starter culture organism complement each other and released the free amino acids The maximum proteolysis was recorded

when the ratio of thermophilus to bulgaricus was 1 1 (Frank and Marth 1988)

Hickey et al (1983) reported that the release of amino acid threonine was essential for the production of acetaldehyde L bulgaricus possessed the enzyme threonine aldolase which was found to convert threonine to acetaldehyde

Abraham et al (1993) pointed out that proteolytic system of L delbrueckii subsp bulgaricus was very complex Proteinases of the bulgricus were associated with cell wall and were regulated by temperature and growth phase It was observed that the specific proteolytic activity (SPA) was maximum when bulgaricus was grown at 34 to 38°C in milk with low soluble nitrogen Increasing the temperature above 40°C decreased the SPA

Bifidobacteria were reported to be weakly proteolytic and it was found that B bifidum require 60 to 90 hours at 37°C for proteolysis and formation of amino acids (Kurmann, 1988)

2 8 1 Products of proteolyis

Alm (1982c) reported that non protein nitrogen (NPN) content of yogurt as 45 mg per 100 g while Shankar et al

(1983) found that NPN amounted to 7 8 per cent of total nitrogen in yogurt

Sharma and Singh (1982) found that the tyrosine value in fermented skim milk was higher when the pure culture of bulgaricus was grown in skim milk than in mixed culture with thermophilus Tyrosine values in skim milk were 0 41 mg per gram for mixed culture after 18 hours of incubation at 37°C

The soluble NPN content in yogurt was reported to be 50 per cent higher than that in original milk (Deeth 1984)

Tamime and Robinson (1985) reported that free amino acids in milk varied from 3 29 to 10 31 and in yogurt 18 77 to 33 06 mg per 100 ml

Abou Donia et al (1984) found that the tyrosine value of commercial samples of yogurt (Zabadi) ranged from 28 to 48 microgram per ml

Prasad (1990) reported an increase in the content of NPN and tyrosine value in yogurt prepared from raw milk stored for 72 hours at 4°C The content of NPN was 76 5 mg per 100 g and tyrosine value 0 46 mg per g in the products prepared from stored milk The values of NPN and tyrosine in the yogurt from fresh milk were 20 mg per 100 g and 0 29 mg per g

respectively These increase was attributed to presence of psychrotropic bacterial enzymes in the stored milk

Gassem and Frank (1991) studied the pattern of proteclysis in yogurt when the mix was incorporated with enzymes protease A protease B or plasmin Proteolysis was studied on the basis of release of free amino groups (FAG) The enzymes were added into the yogurt mix and aged for 4-5 hours at 37°C After aging the yogurt culture was inoculated Concentrations of FAG were recorded before and after enzyme treatment and also after fermentation The FAG content of ycgurt mix (before fermentation) without enzyme treatrent was 5 8 micromoles per ml The concentration of FAG in yogurt mix (before fermentatien) were increased to 7 1-8 4 micromoles per ml due to enzyme treatment There was further increase in FAG content after the fermentation The FAG content in untreated yogurt protease A Protease B and plasmin treated yogurt were 11 9 14 5 17 4 14 9 micromoles per ml respectively

2 9 Flavour compounds in yogurt

Pette and Lolema (1950c) reported that the acetaldehyde and other unknown compounds were responsible for typical yogurt flavour

Bottazzi and Vescovo (1969) isolated 84 different strains of thermophilic lactobacilli and five strains of

thermophilic streptococci from French Swiss, German and Italian yogurt They found that there were great variation in quantity of acetaldehyde and acetone produced by the different strains Strains of Lactobacilli were divided into three groups on the basis of quantity of acetaldehyde and acetone produced Group A which constituted 22 6 per cent of the strains studied produced more acetone (maximum 7 2 ppm) than acetaldehyde (0 1 2 3 ppm) Group B (25 2 per cent of total strains studied) produced 3 88 ppm of acetaldehyde and 2 44 ppm of acetone Acetaldehyde and acetone produced by group C were 8 50 ppm and 3 00 ppm respectively The majority of the strains under study (52 2 per cent) belonged to group C It was further indicated that the products with eight ppm acetaldehyde gave typical yogurt flavour All the strains of lactobacilli produced only traces of acetoin and did not produced diacetyl Different strains of thermophilic streptococci studied by Bottazzi and Vescovo (1969) produced 1 7 to 2 3 ppm acetaldehyde 1 2 to 5 2 ppm acetone and 1 5 to 3 0 ppm acetoin

Kosikowski (1977) reported that the ideal flavour in plain yogurt was developed at pH 4 4

Rasic and Kurmann (1978) found that the ratio between acetaldehyde to acetone should be 1 1 2 8 1 to produce desired flavour in yogurt

Singh et al (1982) reported that a mixed culture of thermophilus and bulgaricus produced higher amount of acetaldehyde than individual culture It was also found that the maximum quantity of acetaldehyde (28 per cent) in skim milk inoculated with mixed yogurt culture was produced after 12 hours of incubation at 37°C Prolonged incubation reduced the concentration of the flavour compound

It was also observed that incorporation of L acidophilus along with thermophilus and bulgaricus reduced the concentration of acetaldehyde in skim milk incubated for 18 hours at 37°C (Sharma and Singh 1982)

Manjunath et al (1983) reported that the concent ration of acetaldehyde in yogurt prepared from cow milk was higher than in goat milk yogurt with similar total solid percentage The concentration of acetaldehyde was 150 and 124 microgram per 20 g of yogurt prepared from cow and goat milk respectively

Tamime and Robinson (1985) reported that the typical yogurt flavour was basically due to combination of lactic acid and carbonyl compounds such as acetaldehyde acetone acetoin and diacetyl Certain volatile and non volatile compounds for example pyruvic acid oxalic acid formic acid acetic acid propionic and butyric acid also contributed to flavour

development in yogurt Certain amino acids such as serine glutamic acid proline valine leucine isoleucine and tyrosine did not directly involved in flavour production but they were known as precursors for carbonyl compounds

Shukla et al (1986) studied the effect of incorporation of gelatin pectin gum acacia sodium hexa-meta-phosphate (SHMP) sodium alginate and carboxy-methyl-cellulose (CMC) on diacetyl production in yogurt prepared from buffalo milk Diacetyl content in yogurt with different levels of fat were also investigated They found that the presence of the stabilizers decreased the concentration of diacetyl In standardized buffalo milk yogurt (fat 4 5 per cent) the diacetyl concentration was 10 20 10 85 ppm It was reduced to 9 97-10 46 ppm due to addition of different stabilizers Decreasing the fat percentage also adversely affected the diacetyl level in the product Diacetyl content in yogurt from toned milk (fat 3 0 per cent) was 8 02-8 23 ppm and from double toned milk (fat 1 5 per cent) it was 6 54 6 85 ppm

McGregor and White (1987) used different types of sweetening agents in yogurt to study their effect on flavour producing compound They compared sucrose alone (added at the rate of four per cent in mix) with combination of sucrose and corn sweeteners (at two per cent each) and corn sweeteners as

a sole source (four per cent) The diacetyl content in the product ranged from 1 1 to 1 5 ppm ard acetaldehyde 24 9 to 27 2 ppm They found that types of sweeteners did not have any significant effect on diacetyl and acetaldehyde concentration in the yogurt

Diacetyl and volatile acidity in yogurt were found to be increased by supplementation of Leuconostoc cremoris and L dextranicum (Prasad and Srinivas 1987) Diacetyl content was increased from 0 0 25 ppm (in control yogurt) to 2 0 4 5 ppm by addition of Leuconostoc specie The concentration of acetaldehyde however decreased from 30 ppm (in control) to 24 1 ppm by the supplementation

Kang et al (1988) reported that optimum flavour and aroma in yogurt was found when acetaldehyde concentration was 23 to 41 ppm at pH 4 4 to 4 0 By the use of gas chromatographic method they found that there was an increase in acetaldehyde and acetic acid content and a decrease in diacetyl after 10 days of refrigerated storage of the product

Marranzini et al (1989) reported that both yogurt culture bacteria were capable of producing acetaldehyde from threonine by symbiotic growth at 42°C High threonine and low glycine levels gave increased acetaldehyde concentration The

reverse ratio of the two amino acids in yogurt decreased the production acetaldehyde

Storing the raw milk at 4°C for 48 hours prior to yogurt preparation increased the diacetyl concentration and decreased the acetaldehyde in yogurt (Prasad 1990) The content of diacetyl in control yogurt was 5 7 ppm The concentration was increased to 6 5 ppm in yogurt prepared from stored milk On the contrary acetaldehyde content was decreased from 26 37 ppm to 23 27 ppm in product prepared from stored milk

2.9.1 Production of the flavour compounds by bifidobacteria

Alm (1982a) reported that acetic acid in milk fermented by bifidobacteria was 650 mg per 100 g The plain yogurt produced 10 mg of acetic acid per 100 g The refrigerated storage of the products did not have any influence on the quantity of acetic acid

Marshall et al (1982) used ultrafiltered cheese whey concentrate and amino acid threonine for fortification in skim milk Fermented products were prepared with B bifidum, B infantis B adolescentis and B longum using two per cent inoculum After incubation for 24 hours at 37°C the acetaldehyde concentration was 27 to 39 ppm The product exhibited typical yogurt texture and flavour

Desjardins et al (1990) studied the rate of lactic acid and acetic acid production by different strains of bifidobacteria in skim milk They reported that the growth and acid production greatly varied amongst the different strains The fast growing strains produced more titratable acidity than slow growing after eight hours of incubation A wide range in acetate-lactate ratio was also observed Only five out of 19 strains studied had a ratio of 1 5 Bifidobacterium bifidum 11147 produced 2 5 times more lactate than acetate and B infantis 27920 three times more acetate than lactate

Murti et al (1992) reported that incorporation of bifidobacteria with normal yogurt starter culture reduced the diacetyl and acetaldehyde concentration in the product The content of acetaldehyde was reduced to six ppm as against 30 95 ppm in control yogurt Similarly diacetyl concentration was also reduced to 0 25 ppm

Ventling and Mistry (1993) studied the production of lactic acid and acetic acid by B bifidum and B longum in ultrafiltered milk They found that there was an increase in acetic acid and lactic acid production in ultrafiltered milk The acetic acid concentration was increased by 2 5 times and lactic acid by 2 9 times in skim milk concentrated by ultrafilteration and fermented by B bifidum

2 10 Organoleptic characteristics of yogurt

The quality of yogurt is usually measured in terms of flavour body and texture The product performance is reflected by the consumer s acceptance Wheying off and weak gel formation in yogurt were considered as serious defects (Chambers 1979)

Seitz (1990) defined the flavour as the combination of taste and odour that is influenced by sensation of contact heat cold and irritation It may be thought as a unitary experience that arises through the stimulation of senses of taste smell and feel

According to Tobias (1990) the term flavour includes sensory properties as watery rich creamy smooth and astringent Both tactual and even visual characteristics are included in general concept of flavour

In the opinion of Norling (19 9) the popularity of yogurt was not only due to its nutritional quality but due also to its typical flavour characteristic texture attractive appearance and different packaging systems

Francis and Ann (1978) suggested that to improve the appearance of acidophilus milk and other fermented product

they should be homogenized after coagulation at a pressure of 50-80 kg per cm^2

Broome et al (1982) reported that replacement of non fat dry milk (NDM) by ultrafiltered cheese whey concentrate at 25 per cent did not affected the taste and texture of yogurt

Labropoulos et al (1982) conducted an experiment on sensory evaluation of fermented milk products made from single and mixed culture of thermophilus bulgaricus and L acidophilus Body and flavour score for yogurt from mixed culture were higher than that of prepared from using individual cultures The product with pure bulgaricus culture was bitter and that of with pure thermophilus was having mild acid flavour Incorporation of L acidophilus as a supplementary culture in yogurt produced a different flavour which was appreciated by the consumers

Manjunath et al (1983) compared the organoleptic characteristics of yogurt prepared with cow and goat milk It was indicated that the characteristic goaty smell was completely masked by the acetaldehyde and finished product had a delicate aroma The average organoleptic score for yogurt with goat milk was 16 as against 15 for cow milk yogurt (The judgement was made on arbitrary 20 point scale) Some members

of panel preferred the product with goat milk and some failed to distinguish it from that of cow milk

Guirguis et al (1984) reported that the fortification of whey protein concentrate (WPC) to replace part of skim milk solids in yogurt improved the viscosity and reduced susceptibility to syneresis

Prasad and Srinivas (1987) found that there was ar increase in organoleptic score for body and flavour of yogurt when the product was prepared with supplementation of Leuconostoc cremoris or L dextranium along with yogurt culture

Tamime and Robinson (1988) reported that excessive numbers of bifidobacteria in fermented product produced a vinegar taint and a poor flavour

Kurmann (1988) suggested that to overcome the mild flavour and insipid taste of the fermented milk, cultured with bifidobacteria, it should be grown with yogurt starter culture

In a study on the influence of incubation temperature on the sensory characteristics of set and stirred yogurt Cho-Ah-Ying et al (1990) found that the panalists preferred the flavour and texture of set yogurt prepared at 38°C than at

43°C The texture of the set yogurt was significantly coarser at 43°C They attributed this to accelerated aggregation of casein micelles resulting in coarse protein net work at higher temperature

Rash (1990) suggested that milk for preparation of fermented product should be wholesome and fresh Any potential flavour defect in raw milk were accentuated during fermentation The most common defect in yogurt was the absence of typical flavour resulting from inadequate acid development Other defects like unclean rancid and bitter occurred from poor quality milk or contamination of starter

Prasad (1990) reported that storing raw milk under refrigerated conditions for 72 hours reduced the body and texture scores from 4 23 to 4 03 and flavour scores from 8 37 to 8 00 for yogurt Pasteurization or activation of Lactoperoxidase system of the milk prior to storage of milk for 72 hours did not adversely affect the body texture and flavour of yogurt prepared from the stored milk Deterioration of flavour in yogurt from stored milk was attributed to the presence of proteolytic enzymes synthesized by the psychrotrophes during storage

Abd El Salam et al (1991) found that fortification of ultrafiltered whey protein concentrate at the rate of 20 per

cent in yogurt prepared from buffalo milk improved taste body and texture and mouth feel of the product

Gassem and Frank (1991) reported that yogurt made from milk treated with protease enzyme from Psudomonas was more firm had greater apparent viscosity and greater syneresis than that of control (untreated)

Hamzawi and Kamaly (1992) found that enrichment of stirred yogurt with cooked wheat grains improved the flavour body and texture score however, the appearance of the product was not liked by the panelists

2 11 Organoleptic characteristic of frozen yogurt

Opdahl and Baer (1991) used whey protein concentrate to replace non-fat dry milk (NDM) for preparation of frozen yogurt The product was subjected to consumer s acceptance test Data from 1005 consumers was studied Amongst the three flavouring products vanilla strawberry and chocolate 95 per cent of consumers preferred the frozen yogurt with strawberry flavour

Mashayekh and Brown (1992) conducted a sensory evaluation test for cultured ice cream fermented with yogurt starter culture They found that the most preferred product on the basis of appearance texture mouth feel and flavour

was the one having a pH of 4 9 incomparison to the product with pH 4 4 and pH 5 4 The members of panel preferred cultured ice cream over the commercial frozen yogurt because of richness and the texture of the former product

Frozen fermented ice cream was prepared by using the mixed starter culture of B bifidum and L acidophilus Sensory evaluation of the product was carried out at different pH The judges were asked to indicate their preferences for the products having the pH of 5 0 5 5 and 6 0 The fermented ice cream with the pH 5 5 was most preferred by the evaluators (Hekmat and McMohan 1992)

MATERIALS AND METHODS

MATERIALS AND METHODS

3 1 Materials

3 1 1 Milk

Fresh pooled cow milk was collected from University livestock farm Mannuthy Thrissur

By partial skimming the fat content of the milk was adjusted to three per cent

3 1 2 Cottage cheese whey

Rennet coagulated fresh cottage cheese whey was collected from Kerala Agricultural University Dairy plant Mannuthy Thrissur

The whey was immediately heated to 90°C for 10 minutes to inactivate residual rennet and bacterial cultures

3 1 2 1 Method of condensation

The cheese whey was condensed to approximately 8 1 concentration by vacuum evaporator Anhydro Type Lab E W O 1688 at a temperature of 45°C with a vacuum of 70 cm of mercury

The condensed whey was immediately cooled to 5°C and stored at -20°C until it was utilized for fortification of yogurt mix The condensed whey was used within a week of its manufacture

3 1 2 2 Preparation of size reduced whey protein dispersion

The whey protein dispersion was prepared by the method described by Le-Lievere (1990)

Part of the condensed whey was heated to 95°C for 10 minutes and acidified with one per cent citric acid to a pH of 4 6 The suspension was centrifuged at 2500 rpm for 15 minutes The sediment of protein was collected after discarding the clear supernatent The whey protein dispersion was immediately used in yogurt mix

Average composition of NDM condensed whey and whey protein dispersion used for fortifiction of yogurt mix is given in Table 3 1

3 1 3 Starter cultures

Freeze dried cultures of (i) S salivarius subsp thermophilus NDRI YH-S and (ii) L delbrueckii subsp bulgaricus YH-L were obtained from National Collection of Dairy Cultures National Dairy Research Institute Karnal

Table 3 1 Average composition of non fat dry milk (NDM) condensed cottage cheese whey and whey protein dispersion used for the fortification of yogurt mix under different treatments

	Total solids	(on dry matter basis)			
		Protein	Fat	Lactose (by difference)	Ash
	(per cent)				
NDM	96 30	35 92	0 83	55 78	7 47
Condensed cottage cheese whey	40 66	10 32	0 00	78 62	11 06
Whey protein dispersion	50 37	15 85	0 00	76 21	7 94

Lyophilised culture of B bifidum 2715 was procured from National Collection of Food Bacteria Agricultural and Food Research Council (AFRC) Institute of Food Research, Reading

3 1 3 1 Maintenance of starter cultures

Pure cultures of thermophilus and bulgaricus were maintained separately in sterile skim milk and subcultured at weekly interval

The starter culture of B bifidum was maintained in sterile skim milk fortified with one per cent dextrose and 0 1 per cent yeast extract Sub-culturing was done at weekly interval

The starter cultures were tested periodically for their purity and activity

3 2 Plan of experiment

To study the effect of incorporation of different levels of whey solids and whey protein dispersion as a substitute for NDM and to assess the growth characteristics of B bifidum along with normal yogurt culture and their effect on biochemical and organoleptic qualities of set and frozen yogurt the following plan of experiment was designed and it is depicted in Fig 1

TREATMENTS

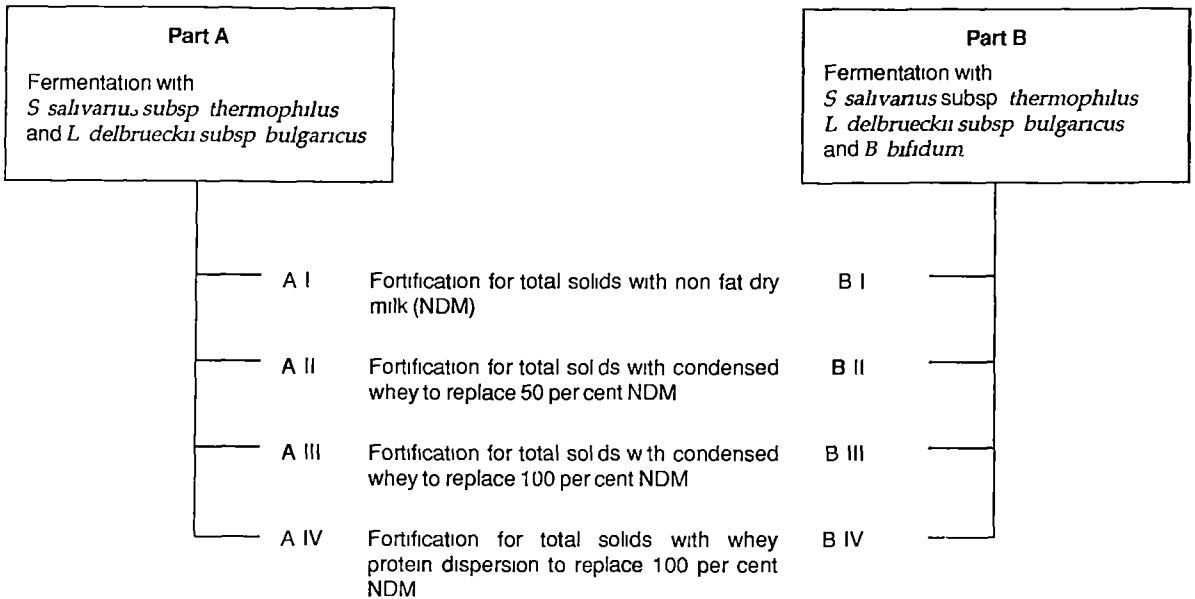


Fig 1 Plan of experiment

On the basis of the starter cultures used for fermentation the experiment was divided as part A and part B

For the treatments under part A, normal yogurt culture viz S salivarius subsp thermophilus and L delbrueckii subsp bulgaricus were used for fermentation

For the treatments under part B the fermentation was carried out by a combination of normal yogurt culture and B bifidum

On the basis of the nature of fortification of milk solids in yogurt mix the part A and part B were divided into four treatments each as described hereunder

Fat and total solids contents of yogurt mix under each treatments of part A and B were standardized to three and 16 per cent respectively

Part A

Fermentation with normal yogurt culture viz S Sal varius subsp thermophilus and L delbrueckii subsp bulgaricus

Treatment A-I (control) Fortification of yogurt mix with NDM

Treatment A-II — Fortification of the mix with condensed whey to replace 50 per cent NDM on dry matter basis

Treatment A-III — Fortification of the mix with condensed whey to replace 100 per cent NDM on dry matter basis

Treatment A-IV — Fortification of the mix with whey protein dispersion to replace 100 per cent NDM on dry matter basis

Part B

Fermentation with a combination of normal yogurt culture and B bifidum

Treatment B I — Fortification of yogurt mix with NDM

Treatment B-II — Fortification of the mix with condensed whey to replace 50 per cent NDM on dry matter basis

Treatment B-III — Fortification of the mix with condensed whey to replace 100 per cent NDM on dry matter basis

Treatment B-IV Fortification of the mix with whey protein dispersion to replace 100 per cent NDM on dry matter basis

3.3 Preparation of yogurt

The yogurt mixes under all the treatments of part A and part B were used for the preparation of yogurt

After the standardization of fat and total solids sugar at the rate of five per cent (w/v) was added into the mix

The yogurt mix was pre heated to 70°C and homogenized in a two stage homogenizer at 2000 and 500 PSI

After the homogenization the mix was heated to 85°C for 30 minutes and subsequently cooled to 30°C

The mix under all the treatments of part A were inoculated with an active cultures of (i) S salivarius subsp thermophilus YH-S and (ii) L delbrueckii subsp bulgaricus YH-L grown separately and added at the rate of one per cent each

The yogurt mixes under the treatment of part B were inoculated with an active cultures of (i) S salivarius subsp thermophilus YH-S (ii) L delbrueckii subsp

bulgaricus YH-L at the rate of one per cent each and (iii) B bifidum 2715 at the rate of ten per cent The rate of inoculum of B bifidum was decided by a pre-experimental trial

After thorough blending of the cultures with the mix it was dispensed in 50 ml glass containers for set yogurt and 500 ml glass containers for stirred yogurt

The containers were then transferred to an incubator set at 30°C (Tamime and Robinson 1985) The pH of mix was monitored periodically till the desired pH was attained The cups were immediately transferred to a refrigerator maintained at 4°C for cooling

The period of the incubation was adjusted in such a way to get a constant pH of 4 6 after overnight cooling

3 4 Preparation of the frozen yogurt

Stirred yogurt from all the treatments under part A and part B were used as a base for preparation of frozen yogurt

After fermentation and cooling the coagulum of yogurt was broken To this cream sugar sodium alginate (stabilizer) and glycerol monostearate (emulsifier) were added

and thoroughly blended to obtain a mix with the following percentge composition

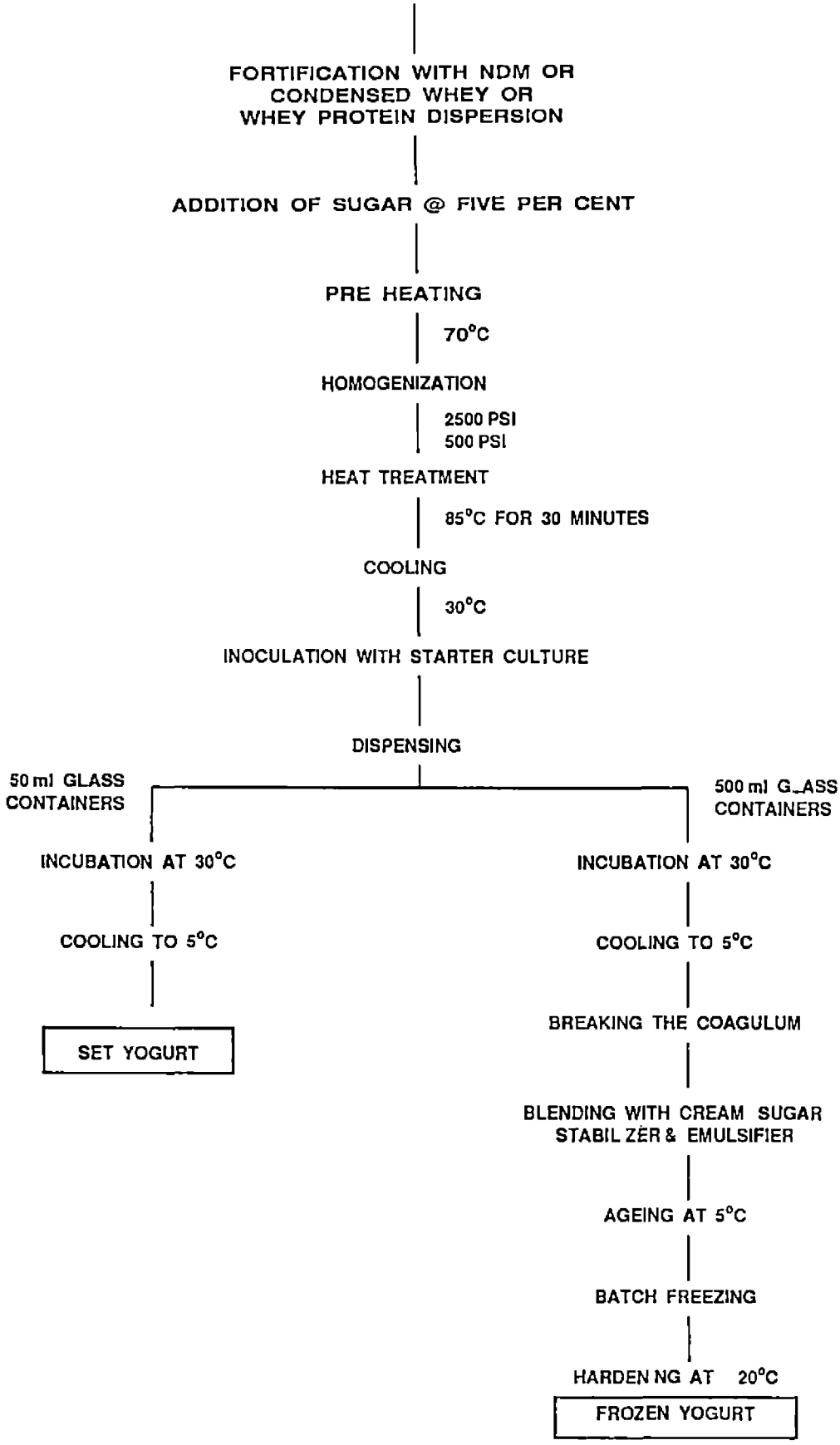
Fat	10
Solid not fat	13-14
Sugar	15
Sodium alginate	0 25
Glycerol monostearate (GMS)	0 25

Fresh cream with 50 per cent fat was used for standardisation of fat in frozen yogurt The mixture of cream sugar sodium alginate and GMS was heat treated at 85°C for 30 minutes and cooled to 30°C before addition into stirred yogurt

After thorough mixing the product was aged at 4°C for a minimum period of five hours

The product was then frozen by using a batch freezer (Vulcan laval) to 40 per cent over run The frozen yogurt was collected in approximately one and half litre stainless steel containers and stored at -20°C for hardening

The preparation of set and frozen yogurt is depicted in the FLOW CHART


FORTIFICATION WITH NDM OR
CONDENSED WHEY OR
WHEY PROTEIN DISPERSION
ADDITION OF SUGAR @ FIVE PER CENT
PRE HEATING
70°C
HOMOGENIZATION
2500 PSI
500 PSI
HEAT TREATMENT
85°C FOR 30 MINUTES
COOLING
30°C
INOCULATION WITH STARTER CULTURE
DISPENSING
50 ml GLASS CONTAINERS
500 ml GLASS CONTAINERS
INCUBATION AT 30°C
COOLING TO 5°C
SET YOGURT
INCUBATION AT 30°C
COOLING TO 5°C
BREAKING THE COAGULUM
BLENDING WITH CREAM SUGAR
STABILIZER & EMULSIFIER
AGEING AT 5°C
BATCH FREEZING
HARDENING AT 20°C
FROZEN YOGURT

3 5 Analysis of set yogurt

Samples of set yogurt from part A and part B under all treatments were collected after cooling The samples were analysed for estimating following microbiological and biochemical parameters

3 5 1 Microbiological analyses

3 5 1 1 Preparation of diluents

Phosphate buffer was used for serial dilution of the samples One ml of the yogurt sample was transferred aseptically into nine ml of sterile phosphate butter Serial dilutions were prepared upto the dilution factor of 10^9 One ml of appropriate dilution (10^6-10^9 for enumération of starter bacteria and 10^1 for yeast and mould count) was used for the bacterial and yeast and mold counts

3 5 1 2 Enumeration of thermophilus and bulgaricus in set yogurt

Yogurt lactic agar (Matalon and Sandine, 1986) was used for enumeration of thermophilus and bulgaricus in the samples of set yogurt under all the treatment of part A and part B One ml sample of appropriate diluentions (10^6-10^9) was transferred into sterile petri dishe in duplicate

Approximately 10 to 12 ml of the melted agar medium was then poured and mixed thoroughly by rotating the petri dishes After the solidification of the media the petri dishes were placed up side down in the incubator set at 37°C under reduced oxygen tension for 48 hours

The colonies of thermophilus and bulgaricus were enumerated separately based on differential colony characteristics as described by Matalon and Sandine (1986) The colonies of thermophilus were smaller white and without any cloudy zone The colonies of bulgaricus appeared as large white and surrounded by a cloudy zone

3 5 1 3 B bifidum count

Lithium chloride-sodium propionate agar (LP agar) (Lapierre et al 1992) with the following composition was used for the enumeration of B bifidum in yogurt

Liver infusion	35 g
Lactose	10 g
Peptone	10 g
Sodium chloride	2 g
Lithium chloride	2 g
Sodium propionate	3 g
Agar	20 g
Distilled water	1000 ml

All the ingredients of the medium were dissolved in distilled water and the pH was adjusted to 6 7 ± 0 2 The medium was sterilized at 121°C for 15 minutes

One ml of the appropriate dilutions (10^6-10^9) of the sample was transferred into the petri dishes in duplicate Approximately 10 to 12 ml of melted agar was poured and mixed thoroughly The plates were incubated at 37°C for 48 hours under reduced oxygen tension White smooth glistening convex colonies of B bifidum were counted Sample of all the treatment under part B were used for enumeration of B bifidum

The results of starter counts were expressed as colony forming unit (cfu) per ml x 10^9

3 5 1 4 Coliform count

The samples of set yogurt under the treatments of part A and B were enumerated for the coliform by using violet red bile agar (IS 5401 1969)

3 5 1 5 Yeast and mold count

Yeast and mold in set yogurt under the treatments of part A and part B were enumerated by using potato-dextrose agar (IS 5403 1969)

3 5 2 Biochemical analyses of set yogurt

Samples of set yogurt from all the treatment under part A and part B were collected after cooling for estimating the following biochemical parameters

3 5 2 1 Acidity and pH

Titratable acidity of the samples of set yogurt were measured by method prescribed for fermented milk product IS 1479 part II (1961)

pH of the samples during fermentation and after cooling was monitored by an electronic digital pH meter

3 5 2 2 Total solids

Total solid in yogurt was determined by vacuum drying (Tamime and Robinson 1985) Sodium hydroxide was used as a moisture absorber

3 5 2 3 Non protein nitrogen (NPN)

The content of NPN in the samples of set yogurt were estimated by the method of IS 1479 part II (1961)

3 5 2 4 Tyrosine value

The tyrosine values of the samples of set yogurt were determined by the procedure of Lowry et al (1951)

3 5 2 5 Diacetyl content

The procedure of Owades and Jakovac as modified by Pack et al (1964) was adopted for measuring the content of diacetyl in set yogurt

3 5 2 6 Acetaldehyde content

The content of acetaldehyde in the samples of set yogurt were determined by the method developed by Lindsay and Day (1965)

3 5 2 7 Content of acetic acid

The content of acetic acid in the samples of set yogurt were determined by using AIMIL NUCON gas chromatograph series 5700 with the following conditions

Column type		Glass column Chromosorb 101
Column temperature		160°C
Detector temperature		190°C
Carrier gas flow rate		30 ml per minute
Gas pressure	Nitrogen	3 kg per square cm
	Hydrogen	2 kg per cm^2
	Oxygen	1 kg per cm^2
Chart speed		One centimetre per minute

Sample processing

The samples of the set yogurt were double filtered using cotton pad in the first instance and filter paper (Whatman No 1) subsequently to obtain clear filtrate The filtrates were used for detection of acetic acid

The concentration of acetic acid in the samples was determined by measuring the height of the peak and retention time in centimetres Area under the peak was calculated by multiplying height of peak with retention time

A standard curve was prepared with different concentrations of acetic acid

3.6 Sensory evaluation of set yogurt

The samples of set yogurt were evaluated for sensory characteristics by a panel of five judges The score card proposed by Pearce and Heap (1974) was adopted for evaluation Average score obtained from five members of panel for each replication was used for statistical analysis

Score card proforma for evaluation of yogurt is as follows

SCORE CARD FOR YOGURT

Date

Taster

Code No

a Appearance and colour
Defects

b Body and texture
Defects

c Flavours
Defects

Overall score

Judge the three characteristics on the 1 to 5 scale

5 Excellent

4 Very good

3 Good

2 Fair

1 Poor

The overall score was obtained by multiplying the flavour score by two and then adding the score to rest An excellent yogurt gives an overall score of 20

Possible defects

Appearance & colour	Extraneous matter lack of uniformity unnatural colour, surface discolouration wheying off fat separation gassiness
Body and texture	Too thin, gelatinous chalky lumpy or granular slimy
Flavour	Excess acid, excess sugar excess stabiliser excess milk powder yeasty unclean

3 7 Analysis of frozen yogurt

The samples of frozen yogurt stored at -20°C were collected after freezing and hardening (0 day) and also on 15th 30th 45th 60th 75th and 90th day of storage The samples for bacteriological analysis were collected in sterilized glass containers Immediately after drawing the samples were thawed to room temperature and used for analyses of the following parameters

1 Viable count of thermophilus and bulgaricus
2 Viable count of B bifidum
3 pH
4 Acidity
5 Tyrosine value
6 Acetic acid content (only on 0 45th and 90th day)

The analytical procedure for the samples of the frozen yogurt were same as described under 3 5

3 8 Sensory evaluation of frozen yogurt

The samples of frozen yogurt after freezing and hardening (0 day) and on every 15th day upto 90 days were subjected to sensory evaluation by a panel of five judges

The proforma of the score card used for sensory evaluation of frozen yogurt is as follows

SCORE CARD FOR EVALUATION OF FROZEN YOGURT

Date

	Characteristics	Perfect score	Code Nos I	II	III	IV
I	Flavour Defects	45				
II	Body and texture Defects	30				
III	Colour and package Defects	5				
IV	Melting quality Defects	5				
V	Bacteria	15	15	15	15	15
	Total	100				

Possible defects

Flavour	Excess acid excess stabiliser harsh flavours unnatural flavours flat flavours metallic flavours unclean
Body & texture	Crumbly soggy and weak body course texture sandy buttery fluffy texture
Colour & package	Unnatural colour
Melting quality	Curdy meltdown quick melting, very slow melting

Name and Signature

The score card proposed for sensory evaluation of ice cream (Nelson and Trout 1964) with slight modification was adopted for the evaluation of frozen yogurt

According to Nelson and Trout (1964) there was a general practice to allot full rating of 15 points under the item of bacteria as it is impossible to judge the bacterial population by organoleptic test

3 9 Statistical analysis

The experiment was carried out with six replication for all the treatments under part A and part B The data were subjected to statistical analysis (Snedecor and Cochran 1967) The complete randomised design (CRD) was selected to test the significance between the treatments Paired t-tests were used to compare the set yogurt and frozen yogurt and also for comparison of frozen ycgurt at different time intervals

During the study it was noticed that there was a substantial decrease in the count cf L delbrueckii subsp bulgaricus under the treatment B-I (in association with bifidobacteria) in comparison with A-I The count of S salivarius subsp thermophilus however, showed a marginal increase in B-I Further the count of bulgaricus under treatment B-III showed lesser decrease than B-I In order to

closely monitor the count of thermophilus and bulgaricus in association with B bifidum and effect of incorporation of condensed whey on the starter culture a supporting trial with ten replication was carried out The data obtained under the supporting trial was subjected to statistical analysis similar to that of main trial

The mean values of NPN after analysis of variance showed a very close similarity between the treatments of part A and part B Therefore to test the significance between the treatments I II III and IV the data was regrouped according to method of Mead (1988) and analysed for the test of significance

RESULTS

RESULTS

The results obtained in the present investigation are presented under the following subheadings

Microbiological analyses

Biochemical analyses and

Sensory evaluation

Microbiological analyses

The samples of set and frozen yogurt were analysed for viable count of S salivarius subsp thermophilus, L delbrueckii subsp bulgaricus and B bifidum

Two trials were conducted to assess the growth of the starter culture in yogurt under different treatments

The main trial was carried out with six replications The samples of set and frozen yogurt were analysed for the viable count of thermophilus, bulgaricus and bifidobacteria under all the treatments of part A and part B The samples of frozen yogurt stored at -20°C were tested for the viable starter count till the end of storage period of 90 days The values in the main trial with six replication showed a substantial differences in starter counts between the treatments but they were found to be statistically not

significant A supporting trial therefore was carried out for the viable count of thermophilus bulgaricus and bifidobacteria in set and frozen yogurt under treatments A-I A-III B-I and B-III with ten replications

The samples of frozen yogurt stored at -20°C in supporting trial were analysed for viable starter count for 30 days as against the 90 days in main trial

The result obtained for both the trials are presented hereunder

4 1 Viable count of starter bacteria in set yogurt under different treatments (Main trial)

The mean value of S salivarius subsp thermophilus L delbrueckii subsp bulgaricus and B bifidum count under different treatments are presented in Table 4 1a

The thermophilus count under A I, A-II A-III and A-IV were 3 23 x 10^9 3 66 x 10^9 3 93 x 10^9 and 3 21 x 10^9 cfu per ml respectively Slight increase in thermophilus count was recorded under treatments A II and A-III than A-I (control) But these difference were statistically not significant (Table 4 1b) The count under A IV was almost equal to A I The thermophilus count under B I B-II B-III and B-IV were 4 26 x 10^9 3 70 x 10^9 3 06 x 10^9 and 3 16 x 10^9 cfu per ml

Table 4 1a Viable count of starter bacteria in set yogurt under different treatments (Main trial)

Treatments	S salivarius subsp thermophilus	L delbrueckii subsp bulgaricus	B bifidum
	(cfu per ml x 10^9)		
A-I	3 23 ± 0 19	3 91 ± 0 41	--
A II	3 66 ± 0 63	3 46 ± 0 56	--
A-III	3 93 ± 0 42	3 65 ± 0 36	-
A-IV	3 21 ± 0 56	2 36 ± 0 26	-
B-I	4 26 ± 0 57	2 40 ± 0 76	2 25 ± 0 45
B-II	3 70 ± 0 49	2 67 ± 0 86	3 36 ± 0 80
B-III	3 06 ± 0 37	3 46 ± 1 24	3 26 ± 0 15
B-IV	3 16 ± 0 75	2 55 ± 0 11	3 48 ± 0 32

Eacn value is the mean of six replications

Table 4 1b Analysis of variance

	Source	DF	MS	F
Count of thermophilus	Between treatment	7	1 10	0 65 NS
	Within treatment	40	1 68	
Count of bulgaricus	Between treatment	7	2 34	0 87 NS
	Within treatment	40	2 69	
Count of bifidobacteria	Between treatment	3	1 91	1 28 NS
	Within treatment	20	1 49	

NS - Not significant

The thermophilus count under B-I was substantially higher than the count under A-I The values under A-II and B-II A-IV and B-IV were very close to each other The differences in the treatments were found to be statistically not significant (Table 4 1b)

The bulgaricus count under treatment A-I, A-II A-III and A-IV were 3 91 x 10^9 3 46 x 10^9 3 65 x 10^9 and 2 36 x 10^9 cfu per ml respectively (Table 4 1a) The corresponding figures under B-I B-II B III and B-IV were 2 40 x 10^9, 2 67 x 10^9, 3 46 x 10^9 and 2 55 x 10^9 cfu per ml respectively The count under the treatments of part B were substantially lower than the corresponding values of part A with the exception of treatment IV (A-IV and B-IV) The maximum difference was noticed between A-I and B I and minimum between A-III and B-III However the difference in bulgaricus count between the treatments were found to be statistically not significant (Table 4 1b)

The bifidobacteria count under treatment B-I B-II B-III and B-IV were 2 25 x 10^9 3 36 x 10^9, 3 26 x 10^9 and 3 48 x 10^9 cfu per ml (Table 4 1a) The count under B-II B-III and B-IV were substantially higher than B-I These differences in the treatments were found to be statistically not significant (Table 4 1b)

The findings of main trial indicated that there was marginal increase in thermophilus count under B-I in comparison with A-I and all the treatments under part B (except B-IV) had lower bulgaricus count than the respective treatments under part A, with substantial decrease in number under B I as compare to A I

It was also found that the decrease in bulgaricus count was lesser under B-III than B-I the difference being statistically not significant

It was therefore found desirable to conduct a supporting trial with increased number of replications The objective of the supporting trial was to closely monitor the thermophilus and bulgaricus count in association with bifidobacteria and possible beneficial effect of incorporation of condensed whey on the starter activity

The second objective of the supporting trial was to substantiate the findings of main trial in respect of thermophilus bulgaricus and bifidobacteria count by increasing the number of replication to ten

The supporting trial comprised of four treatments viz A-I A III B-I and B-III The parameters studied were thermophilus, bulgaricus, bifidobacteria count in set yogurt and frozen yogurt The sample of frozen yogurt made under

supporting trial were also analysed for viable starter count for 30 days of storage period

The result obtained in supporting trial are as under

4 2 Viable count of starter bacteria in set yogurt under different treatments (Supporting trial)

The viable count of thermophilus bulgaricus and bifidobacteria in set yogurt under different treatments for the supporting trial is presented in Table 4 2a

The thermophilus count under A-I and A-III were 3 05 x 10^9 and 3 46 x 10^9 cfu per ml respectively Corresponding values under B-I and B-III were 3 40 x 10^9 and 2 99 x 10^9 cfu per ml respectively A marginal increase in thermophilus count was observed under A-III as compared to A-I Similarly a slight increase in count was recorded under treatment B-I in comparison with A-I The differences in the count between treatments were found to be statistically not significant (Table 4 2b)

The bulgaricus count in set yogurt under treatments A-I, A-III B-I B-III were 2 98 x 10^9 3 29 x 10^9 1 66 x 10^9 and 2 95 x 10^9 cfu per ml The differences in bulgaricus count between A I and A III and also between A-III and B-III were statistically not significant The calculated critical

Table 4 2a Viable count of starter bacteria in set yogurt under different treatments (Supporting trial)

Treatments	S salivarius subsp thermophilus	L delbrueckii subsp bulgaricus	B bifidum
	(cfu per ml x 10^9)		
A-I	3 05 ± 0 10	2 98 ± 0 04	--
A-III	3 46 ± 0 21	3 29 ± 0 18	--
B I	3 40 ± 0 22	1 66 ± 0 05	2 95 ± 0 24
B-III	2 99 ± 0 09	2 95 ± 0 11	2 80 ± 0 18
CD	--	0 51	--

Each value represents the mean of 10 replications

Table 4 2b Analysis of variance

	Source	DF	MS	F
Count of thermophilus	Between treatment	3	0 57	2 01 NS
	Within treatment	36	0 29	
Count of bulgaricus	Between treatment	3	5 23	39 61**
	Within treatment	36	0 13	
Count of bifidobacteria	Between treatment	1	0 11	0 24 NS
	Within treatment	18	0 46	

NS - Non significant

** - Highly significant (P<0 01)

difference (CD) was 0 51 A highly significant (P<0 01) reduction in bulgaricus count was observed under treatment B-I in comparison to A-I The count under treatment B-III was significantly (P<0 01) higher in comparison to B-I (Table 4 2b)

The bifidobacteria count in set yogurt under treatments B-I and B III were 2 95 x 10^9 and 2 80 x 10^9 cfu per ml This difference in the count between treatments was statistically not significant (Table 4 2b)

4 3 Ratio between the starter bacteria in set yogurt (Main trial)

The ratio between the (i) thermophilus-bulgaricus (ii) thermophilus bifidobacteria and (iii) bulgaricus-bifidobacteria were calculated from the values of viable count of starter bacteria The ratio in set yogurt under different treatments (after the incubation and cooling) are depicted in Table 4 3a

The thermophilus-bulgaricus ratio in set yogurt under treatment A-I (control) was 1 21 The ratio under treatments A-II and A-III were near to one The thermophilus-bulgaricus ratio in set yogurt under treatment A-IV was lower (0 74) than that of optimum

Table 4 3a Ratio between the starter bacteria in set yogurt (Main trial)

Treatments	Ratio between		
	thermophilus-bulgaricus	thermophilus-bifidobacteria	bulgaricus-bifidobacteria
A-I	1 21 ± 0 15	--	--
A-II	0 95 ± 0 16	--	--
A-III	0 93 ± 0 13	--	-
A-IV	0 74 ± 0 33	--	--
B-I	0 56 ± 0 10	0 53 ± 0 13	0 94 ± 0 39
B-II	0 72 ± 0 16	0 91 ± 0 21	1 26 ± 0 48
B III	1 13 ± 0 16	1 07 ± 0 26	0 94 ± 0 52
B-IV	0 81 ± 0 43	1 10 ± 0 44	1 37 ± 0 14

Each value is the mean of six replications

Table 4 3b Analysis of variance

Source		DF	MS	F
thermophilus bulgaricus	Between treatment	7	0 65	0 76 NS
	Within treatment	40	0 84	
thermophilus bifidobacteria	Between treatment	3	1 06	2 11 NS
	Within treatment	20	0 50	
bulgaricus bifidobacteria	Between treatment	3	0 32	0 31 NS
	Within treatment	20	1 05	

NS - Non-significant

The thermophilus-bulgaricus ratio in set yogurt under treatments B-I B-II B-III and B-IV were 0 56, 0 72 1 13 and 0 81 The ratio under B-I, B-II and B-IV were lower than the normal and B-III was near to the ratio found in yogurt under treatment A-I (control) Statistical analysis, however revealed that the differences in the ratio between the treatments were not significant (Table 4 3b)

The thermophilus-bifidobacteria ratio under B-I was very low (0 53) in comparison with B-II B-III and B-IV The bulgaricus-bifidobacteria ratio in yogurt under treatment B-I B-II B-III and B-IV were 0 94, 1 26 0 94 and 1 37 respectively (Table 4 3a) The differences in the ratio between the treatments were statistically not significant (Table 4 3b)

4 4 Ratio between the starter bacteria in set yogurt (Supporting trial)

The ratio between the (i) thermophilus-bulgaricus (ii) thermophilus-bifidobacteria and (iii) bulgaricus-bifidobacteria were calculated from the values of viable count of starter bacteria

The ratio in set yogurt (in supporting trial) under different treatment are given in Table 4 4a

Table 4 4a Ratio between the starter bacteria in set yogurt (Supporting trial)

Treatments	Ratio between		
	thermophilus-bulgaricus	thermophilus-bifidobacteria	bulgaricus-bifidobacteria
A-I	0 97 ± 0 03	-	--
A-III	0 95 ± 0 08	-	--
B I	0 48 ± 0 08	0 86 ± 0 14	1 77 ± 0 16
B-III	0 99 ± 0 05	0 93 ± 0 06	0 94 ± 0 06
CD	0 28	--	0 57

Each value is the average of 10 replications

Table 4 4b Analysis of variance

	Source	DF	MS	F
thermophil is bulgaricus	Between treatment	3	0 51	12 93**
	Within treatment	36	0 04	
thermophilus bifidobacteria	Between treatment	1	0 02	0 17 NS
	Within treatment	18	0 12	
bulgaricus bifidobacteria	Between treatment	1	3 19	19 68**
	Within treatment	18	0 16	

NS - Non-significant

** - Highly significant (P<0 01)

The ratio between the population of thermophilus and bulgaricus in set yogurt under treatments A-I A-III B-I and B-III were 0 97, 0 95 0 48 and 0 99 respectively The ratio under B-I was significantly (P<0 01) lower than that of other treatments (Table 4 4b)

The thermophilus bifidobacteria ratio in set yogurt under treatment B-I and B-III were 0 86 and 0 93 respectively This difference in the ratio was statistically not significant (Table 4 4b)

The bulgaricus-bifidobacteria ratio under B-I and B-III was 1 77 and 0 94 respectively The ratio under B-III was significantly (P<0 01) lower than the ratio under B-I (Table 4 4b)

4 5 Effect of freezing and hardening on viable starter count in yogurt under different treatments (Main trial)

The comparative figures of the viable count of thermophilus bulgaricus and bifidobacteria in set and frozen yogurt are presented in Table 4 5a The viable count of the starter bacteria in frozen yogurt were taken after freezing and hardening at -20°C

The maximum thermophilus count in frozen yogurt under the treatments of part A was 2 06 x 10^9 cfu per ml in

Table 4 5a Effect of freezing and hardening on viable starter count in yogurt under different treatments (Main trial)

Treatments	*S salivarius* subsp *thermophilus*			*L delbrueckii* subsp *bulgaricus*			*B bifidum*		
	Mean value$_9$ (cfu/ml x 10^9)		t value	Mean value$_9$ (cfu/ml x 10^9)		t value	Mean value$_9$ (cfu/ml x 10^9)		t value
	Set yogurt	Frozen yogurt		Set yogurt	Frozen yogurt		Set yogurt	Frozen yogurt	
A I	3 23 ± 0 19	1 77 ± 1 24	1 09 NS	3 91 ± 0 41	1 75 ± 0 96	1 84 NS	-		-
A-II	3 66 ± 0 63	1 25 ± 0 58	2 35 NS	3 46 ± 0 56	1 30 ± 0 76	1 91 NS	-		-
A-III	3 93 ± 0 42	2 06 ± 0 86	1 94 NS	3 65 ± 0 36	2 31 ± 0 75	2 16 NS	-		-
A IV	3 21 ± 0 56	0 94 ± 0 14	5 20 **	2 36 ± 0 26	1 32 ± 0 37	2 09 NS	-	-	-
B I	4 26 ± 0 57	2 45 ± 1 44	1 51 NS	2 40 ± 0 76	1 37 ± 0 93	1 34 NS	2 25 ± 0 45	1 54 ± 0 62	0 82 NS
B-II	3 70 ± 0 49	1 80 ± 0 86	2 10 NS	2 67 ± 0 86	1 47 ± 0 53	0 92 NS	3 36 ± 0 80	2 63 ± 0 63	0 66 NS
B III	3 06 ± 0 37	1 36 ± 0 73	2 11 NS	3 46 ± 1 24	2 63 ± 0 73	0 44 NS	3 26 ± 0 15	1 85 ± 0 60	2 47 NS
B IV	3 16 ± 0 75	1 15 ± 0 57	1 91 NS	2 55 ± 0 11	1 43 ± 0 36	3 38 *	3 48 ± 0 32	1 70 ± 0 58	2 24 NS

Table 4 5b Analysis of variance

	Source	DF	MS	F
Count of thermophilus				
Set yogurt	Between treatment	7	1 10	0 65 NS
	Within treatment	40	1 68	
Frozen yogurt	Between treatment	7	2 13	0 44 NS
	Within treatment	40	4 79	
Count of bulgaricus				
Set yogurt	Between treatment	7	2 34	0 87 NS
	Within treatment	40	2 69	
Frozen yogurt	Between treatment	7	1 53	0 51 NS
	Within treatment	40	3 03	
Count of bifidobacteria				
Set yogurt	Between treatment	3	1 91	1 28 NS
	Within treatment	20	1 49	
Frozen yogurt	Between treatment	3	1 40	0 63 NS
	Within treatment	20	2 24	

NS - Non-significant

treatment A-III and minimum was 0 91 x 10^9 cfu per ml in treatment A-IV

Under treatments in part B the maximum and minimum values were 2 45 x 10^9 and 1 15 x 10^9 cfu per ml under B-I and B-IV respectively

The bulgaricus count in frozen yogurt under different treatments was in the range of 1 32 x 10^9 to 2 63 x 10^9 cfu per ml The lowest count was recorded under treatment A-II and highest under B III

The bifidobacteria count in frozen yogurt under treatments B-I B-II B-III and B-IV were 1 54 x 10^9 2 63 x 10^9 1 85 x 10^9 and 1 70 x 10^9 cfu per ml respectively

The differences between the treatments in thermophilus bulgaricus and bifidobacteria count were statistically not significant (Table 4 5b)

Comparison of the values of starter bacterial count between set and frozen yogurt indicate that there was a decrease in the population of thermophilus bulgaricus and bifidobacteria during the process of freezing and hardening to -20°C (Fig 2 3 and 4) The decrease in the population of starter bacteria during the freezing and hardening of the

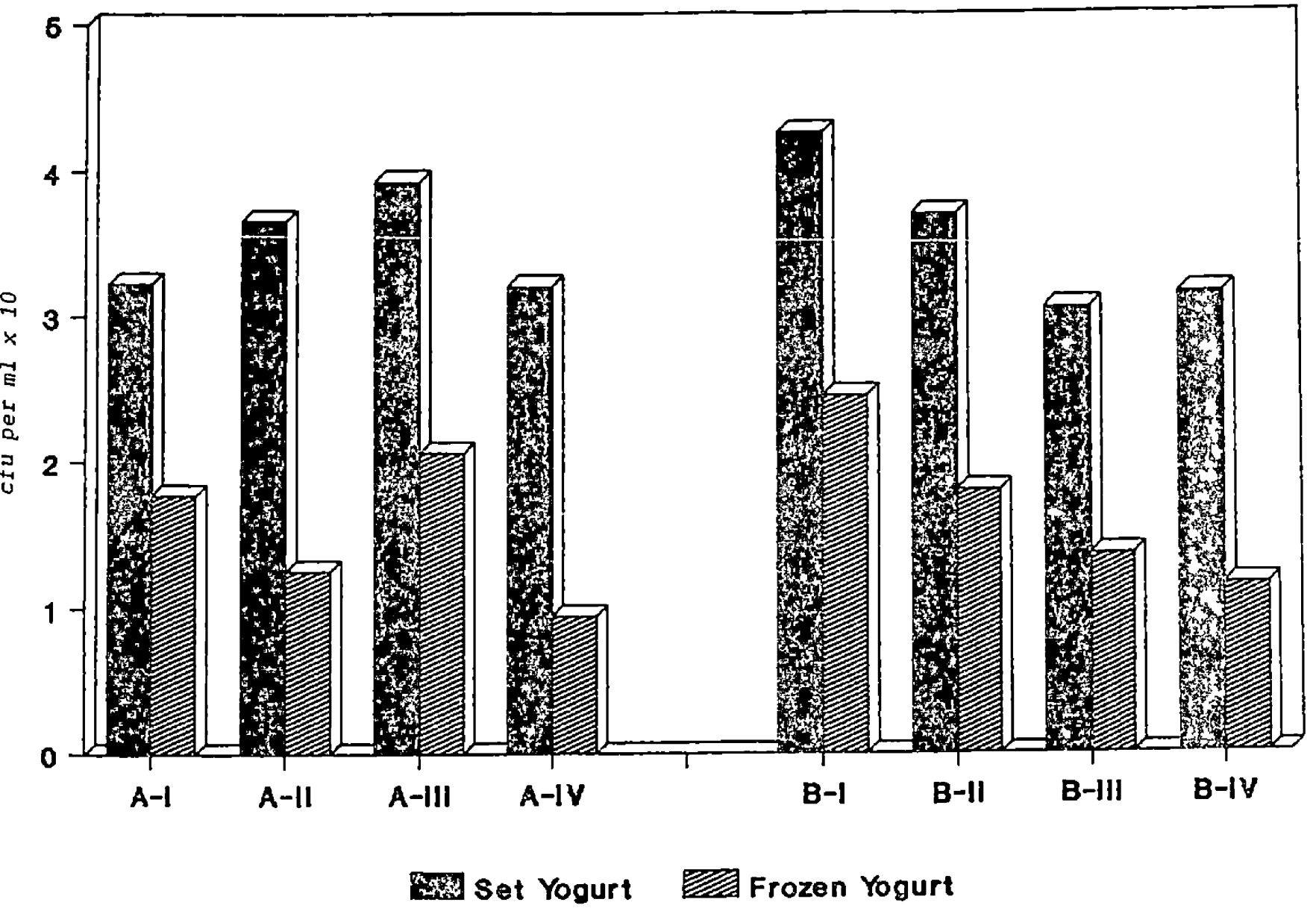


Fig 2 Comparison of S salivarius subsp thermophilus count between set and frozen yogurt under different treatments (Ma n tr al)

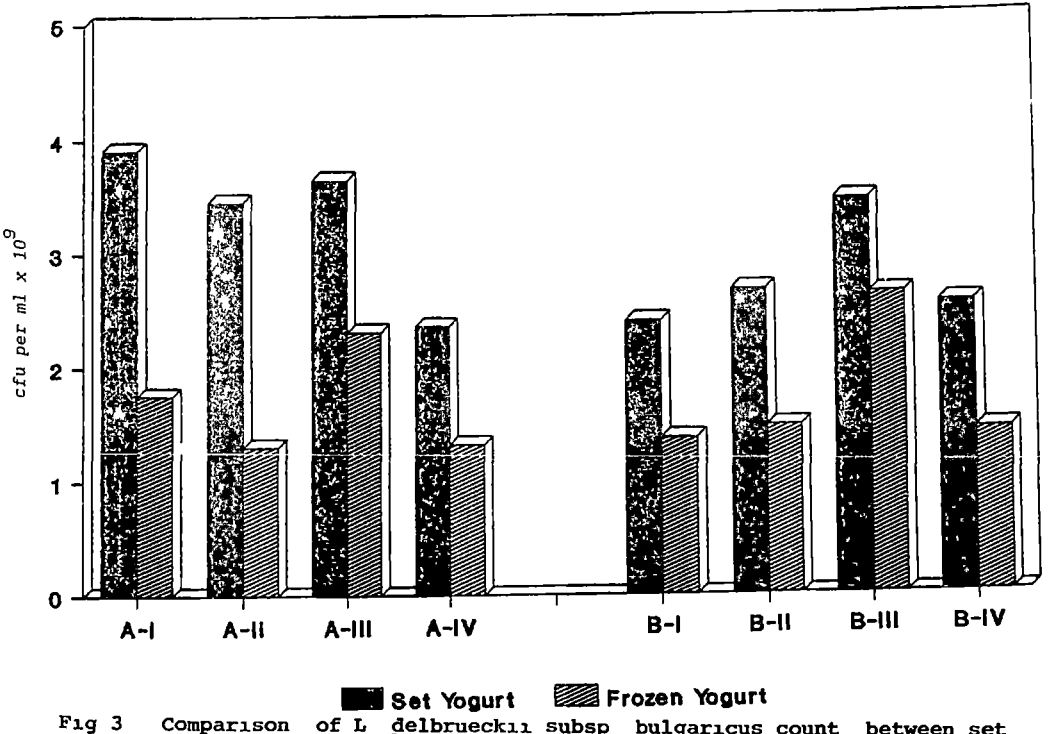


Fig 3 Comparison of L delbrueckii subsp bulgaricus count between set and frozen yogurt under different treatments (Main trial)

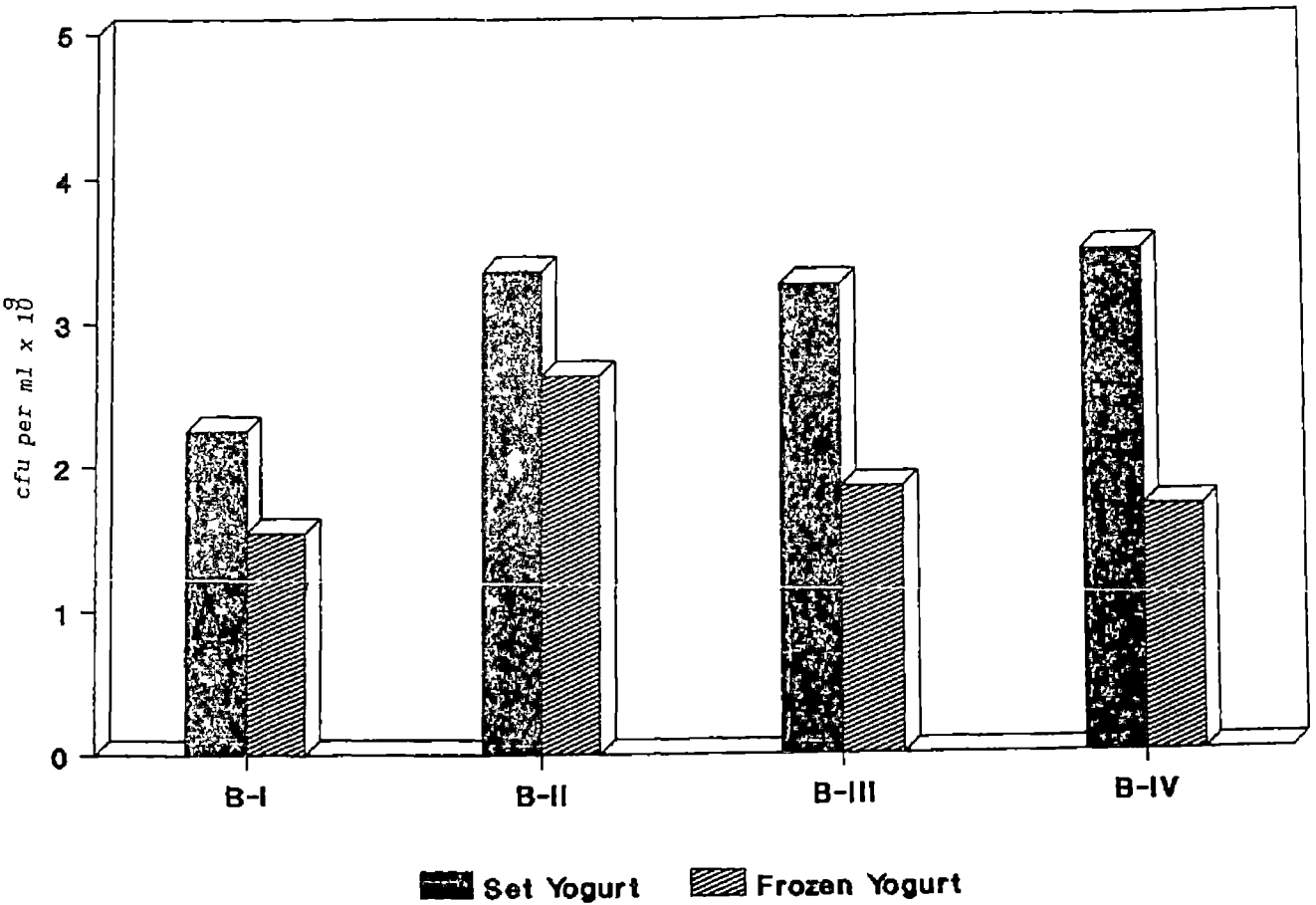


Fig 4 Comparison of B bifidum count between set and frozen yogurt under different treatments (Main trial)

yogurt under different treatment however was less than one logarithmic unit

Paired t test indicate that the decrease in thermophilus count in frozen yogurt was not significant under all the treatment except A-IV Highly significant ($P<0.01$) reduction of thermophilus count was observed under A-IV

Significant reduction of the bulgaricus count was recorded under the treatment B-IV The differences in bulgaricus count between set and frozen yogurt under the remaining treatments were not significant Reduction in bifidobacteria count during the freezing and hardening of yogurt under all the treatment was also statistically not significant

It can be summarised that there were reduction (which was not statistically significant for majority of treatment) in the population of S salivarius subsp thermophilus, L delbrueckii subsp bulgaricus and B bifidum during the freezing and hardening of yogurt The reduction in the population of all three starter bacteria, however, did not exceeded one logarithmic unit

4 6 Effect of freezing and hardening on viable starter count in yogurt under different treatments (Supporting trial)

The comparative figures of thermophilus, bulgaricus and bifidobacteria count in set and frozen yogurt (0 day) are given in Table 4 6a

The thermophilus count in frozen yogurt (0 day) under A-I and A-III were 2 06 x 10^9 and 2 41 x 10^9 cfu per ml respectively The corresponding values under B-I and B-III were 2 15 x 10^9 and 2 43 x 10^9 cfu per ml respectively The differences between the treatments were found to be statistically not significant (Table 4 6b)

The bulgaricus count in frozen yogurt (0 day) under A-I A-III B-I and B-III were 2 27 x 10^9 2 68 x 10^9 1 07 x 10^9 and 2 55 x 10^9 cfu per ml respectively The count under B-I was significantly ($P<0$ 01) lower than A-I, A-III and B-III The bulgaricus count in A-I (control) also significantly lower than A-III (Table 4 6b) The critical difference (CD) was 0 47

The bifidobacteria count in frozen yogurt (0 day) under B-I and B-III were 2 48 x 10^9 and 2 32 x 10^9 cfu per ml respectively The difference between count were found to be statistically not significant (Table 4 6b)

Table 4 6a Effect of freezing and hardening on viable starter count in yogurt under different treatments (Supporting trial)

Treatments	*S salivarius* subsp *thermophilus*			*L delbrueckii* subsp *bulgaricus*			*B bifidum*		
	Mean value (9) (cfu/ml x 10^9)		t value	Mean value (9) (cfu/ml x 10^9)		t value	Mean value (9) (cfu/ml x 10^9)		t value
	Set yogurt	Frozen yogurt		Set yogurt	Frozen yogurt		Set yogurt	Frozen yogurt	
A-I	3 05 ± 0 10	2 06 ± 0 07	5 30**	2 98 ± 0 04	2 27 ± 0 09	7 88**	-	-	-
A-III	3 46 ± 0 21	2 41 ± 0 25	6 56**	3 29 ± 0 18	2 68 ± 0 13	10 08**	-		-
B-I	3 40 ± 0 22	2 15 ± 0 18	4 37**	1 66 ± 0 05	1 07 ± 0 05	9 22**	2 95 ± 0 24	2 48 ± 0 24	4 32**
B III	2 99 ± 0 09	2 43 ± 0 15	3 57**	2 95 ± 0 11	2 55 ± 0 11	6 70**	2 80 ± 0 18	2 32 ± 0 10	2 94*
CD				0 51	0 47				

Each value is the mean of 10 replications

* - Significant (P<0 05) ** Highly significant (P<0 01)

Table 4 6b Analysis of variance

Source		DF	MS	F
Count of thermophilus				
Set yogurt	Between treatment	3	0 57	2 01 NS
	Within treatment	36	0 29	
Frozen yogurt	Between treatment	3	0 34	0 77 NS
	Within treatment	36	0 44	
Count of bulgaricus				
Set yogurt	Between treatment	3	5 23	39 61**
	Within treatment	36	0 13	
Frozen yogurt	Between treatment	3	5 39	49 32**
	Within treatment	36	0 11	
Count of bifidobacteria				
Set yogurt	Between treatment	1	0 11	0 24 NS
	Within treatment	18	0 46	
Frozen yogurt	Between treatment	1	0 13	0 34 NS
	Within treatment	18	0 35	

NS - Non-significant ** - Highly significant (P<0 01)

Comparison of thermophilus (Fig 5) bulgaricus (Fig 6) and bifidobacteria (Fig 7) counts between set and frozen yogurt indicate a highly significant reduction during the process of manufacturing of frozen yogurt The reduction under all the treatment however was limited to less than one logarithmic unit

4 7 Fffect of storage of frozen yogurt at -20°C for 0-90 days on S salivarius subsp thermophilus count under different treatments (Main trial)

The thermophilus count in frozen yogurt under different treatment gradually decreased during the 90 days of storage (Table 4 7a) The rate of decrease was uniform upto 30 days The highest reduction in counts were recorded between 30 and 45 days thereafter rate of reduction was steady till 90 days (Fig 8a and 8b)

Paired t test for comparison of thermophilus count between 0 and 15 0 and 30 0 and 45 0 and 60 0 and 75 0 and 90 days under different treatments was carried out and t values are given in Table 4 7c The perusal of t values indicate that the reduction in the count during storage was statistically not significant

After 90 days of storage the thermophilus count in frozen yogurt under treatments A-I A-II A-III and A-IV were

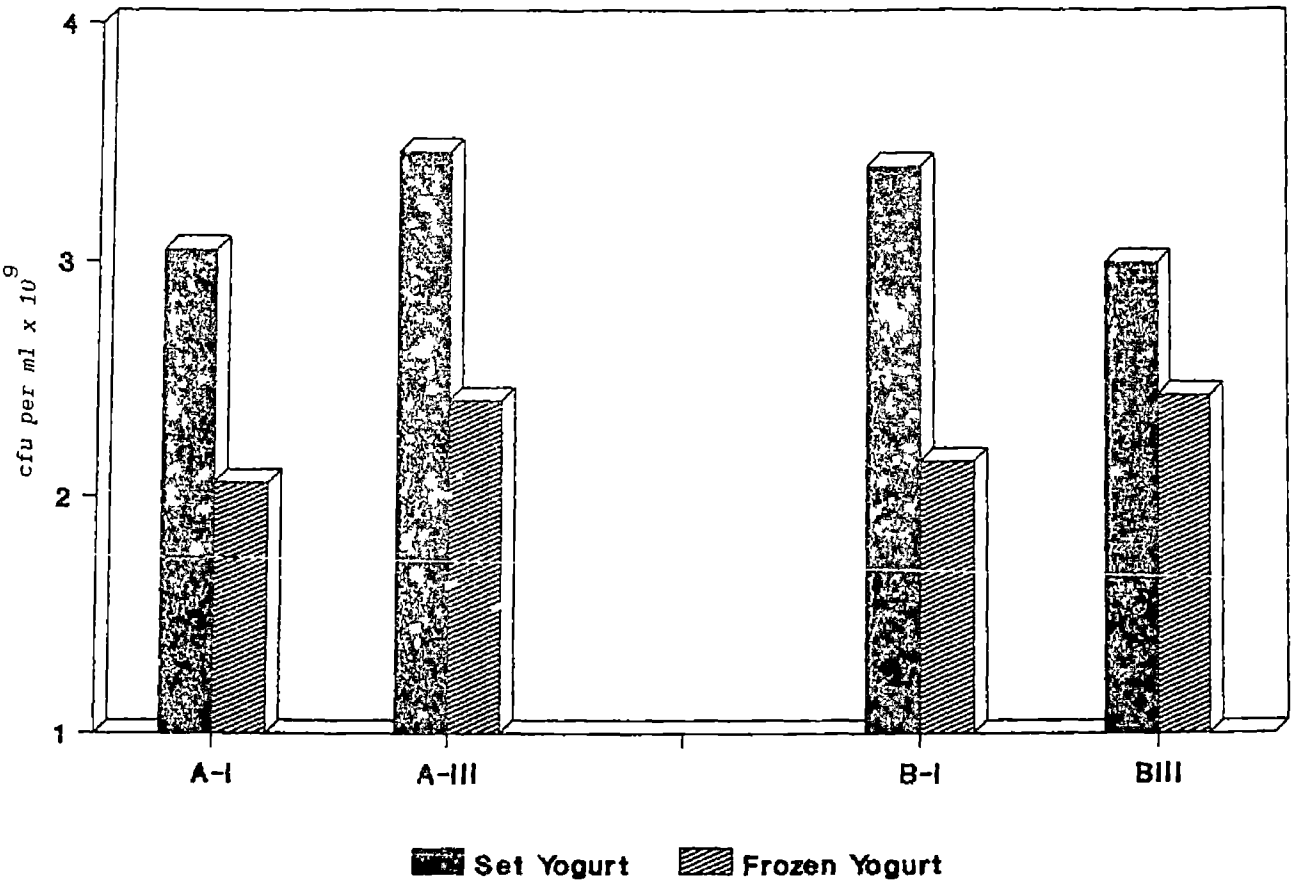


Fig 5 Comparison of S salivarius subsp thermophilus count between set and frozen yogurt under different treatments (Supporting trial)

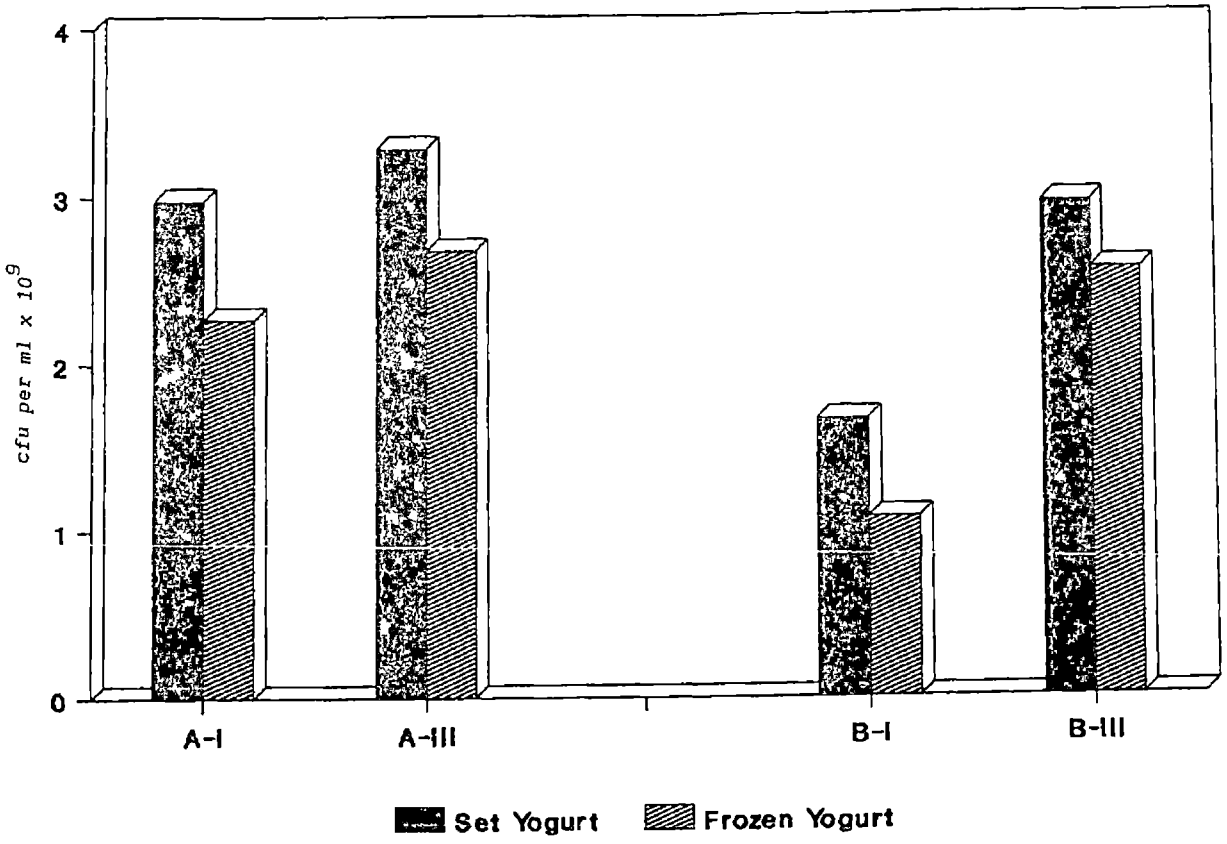


Fig 6 Comparison of L. delbrueckii subsp bulgaricus count between set and frozen yogurt under different treatments (Supporting trial)

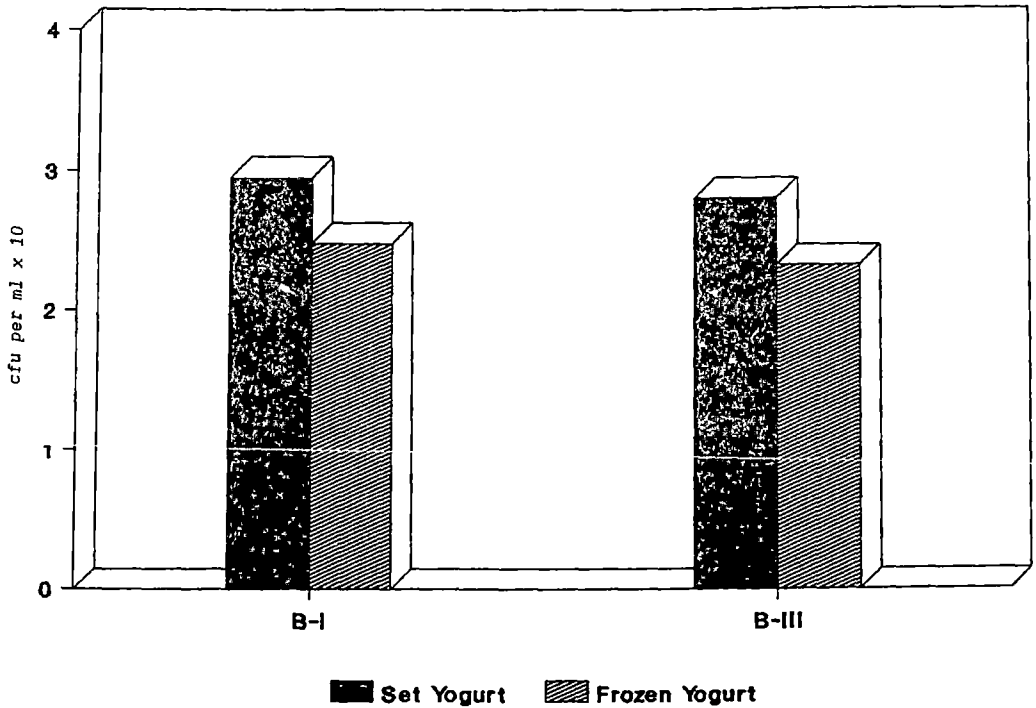


Fig 7 Comparison of B bifidum count between set and frozen yogurt under different treatments (Supporting trial)

Table 4 7a Effect of storage of frozen yogurt at -20°C for 0-90 days on S salivarius subsp thermophilus count under different treatments (Main trial)

Treatment	Period (days) 0	15	30	45	60	75	90
	(cfu per ml x 10^9)						
A I	1 77 ± 1 24	1 08 ± 0 23	1 07 ± 0 62	0 52 ± 0 14	0 47 ± 0 14	0 48 ± 0 09	0 26 ± 0 05
A-II	1 25 ± 0 58	1 13 ± 0 57	1 51 ± 0 62	0 76 ± 0 44	0 24 ± 0 04	0 36 ± 0 05	0 34 ± 0 02
A-III	2 06 ± 0 86	1 11 ± 0 42	1 42 ± 0 67	0 83 ± 0 51	0 36 ± 0 04	0 35 ± 0 04	0 28 ± 0 04
A-IV	0 94 ± 0 14	1 05 ± 0 44	0 31 ± 0 05	0 43 ± 0 08	0 50 ± 0 10	0 37 ± 0 02	0 35 ± 0 04
B-I	2 45 ± 1 44	1 21 ± 0 43	1 13 ± 0 48	0 57 ± 0 25	0 56 ± 0 08	0 45 ± 0 08	0 25 ± 0 03
B II	1 80 ± 0 86	1 27 ± 0 43	0 44 ± 0 08	1 30 ± 0 49	0 47 ± 0 06	0 32 ± 0 06	0 29 ± 0 04
B-III	1 36 ± 0 73	0 87 ± 0 24	0 93 ± 0 57	0 37 ± 0 11	0 57 ± 0 06	0 41 ± 0 07	0 28 ± 0 05
B-IV	1 15 ± 0 57	1 22 ± 0 62	1 44 ± 0 66	0 27 ± 0 06	0 49 ± 0 09	0 48 ± 0 08	0 30 ± 0 05

Table 4 7b Analysis of variance

Period (days)	Source	DF	MS	F
0	Between treatment	7	2 130	0 44 NS
	Within treatment	40	4 790	
15	Between treatment	7	0 090	0 07 NS
	Within treatment	40	1 200	
30	Between treatment	7	1 240	0 74 NS
	Within treatment	40	1 690	
45	Between treatment	7	0 660	1 06 NS
	Within treatment	40	0 620	
60	Between treatment	7	0 070	1 57 NS
	Within treatment	40	0 050	
75	Between treatment	7	0 020	0 78 NS
	Within treatment	40	0 030	
90	Between treatment	7	0 009	0 76 NS
	Within treatment	40	0 011	

NS - Non-significant

Table 4.7c t values

Treatments	0 Vs 15	0 Vs 30	0 Vs 45	0 Vs 60	0 Vs 75	0 Vs 90
A-1	0.48 NS	0.44 NS	0.96 NS	0.97 NS	1.01 NS	1.22 NS
A-II	0.33 NS	0.33 NS	0.69 NS	1.72 NS	1.58 NS	1.53 NS
A-III	0.55 NS	0.55 NS	1.09 NS	1.92 NS	1.99 NS	2.07 NS
A-IV	1.20 NS	1.20 NS	0.53 NS	0.16 NS	1.08 NS	1.05 NS
B-I	0.82 NS	0.83 NS	1.28 NS	1.27 NS	1.39 NS	1.53 NS
B-II	1.50 NS	1.50 NS	0.44 NS	1.60 NS	1.64 NS	1.37 NS
B-III	0.40 NS	0.40 NS	1.28 NS	1.02 NS	1.35 NS	1.48 NS
B-IV	0.54 NS	0.54 NS	1.63 NS	1.04 NS	1.12 NS	1.45 NS

NS - Non-significant

NJ

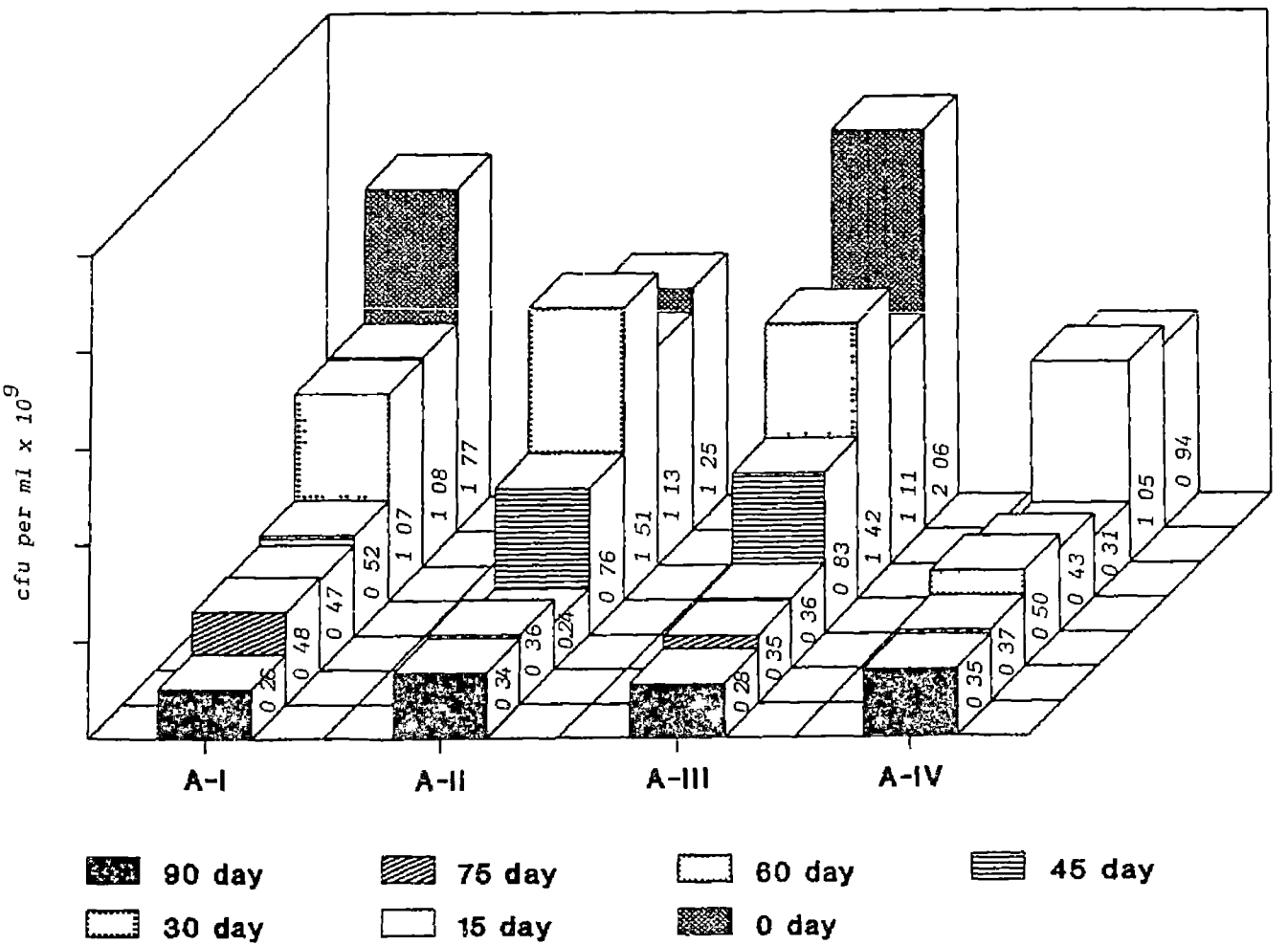


Fig 8a Effect of storage of frozen yogurt on S salivarius subsp thermophilus count under different treatments of part A (Main trial)

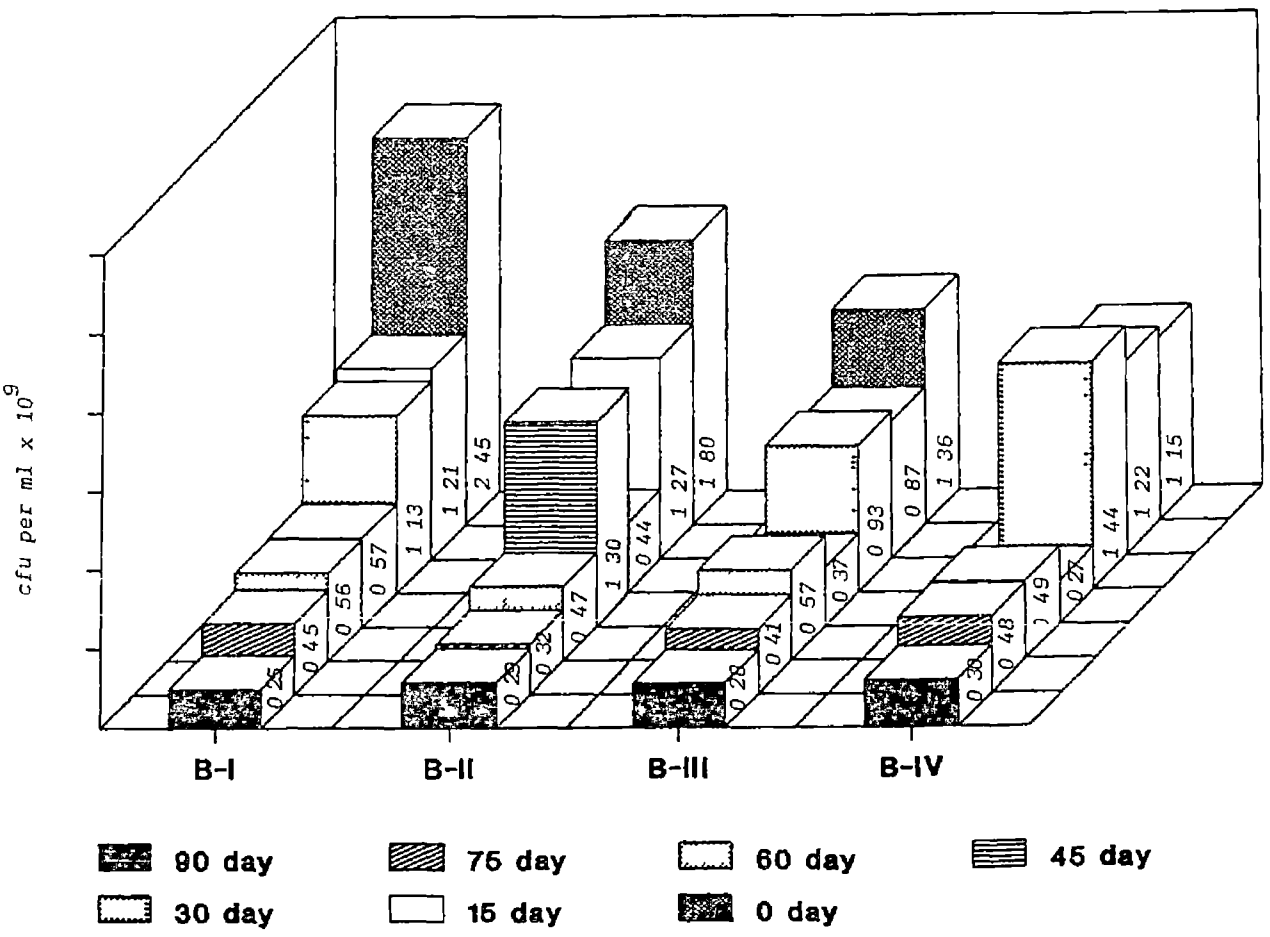


Fig 8b Effect of storage of frozen yogurt on S salivarius subsp thermophilus count under different treatments of part B (Main trial)

0 26 x 10^9 , 0 34 x 10^9 0 28 x 10^9 and 0 35 x 10^9 cfu per ml respectively The corresponding values under B-I B-II B-III and B IV were 0 25 x 10^9 0 29 x 10^9 0 28 x 10^9 and 0 30 x 10^9 cfu per ml respectively Compar son of the thermophilus count in frozen yogurt between 0 and 90 days indicate that the reduction in the counts was limited to one logarithmic unit

4.8 Effect of storage of frozen yogurt at -20°C for 0-90 days on L delbrueckii subsp bulgaricus count under different treatments (Main trial)

The bulgaricus count in frozen yogurt under different treatments stored at -20°C for 0-90 days are presented in Table 4 8a

The bulgaricus count in frozen yogurt under different treatments gradually decreased during the storage (Fig 9a and 9b) The rate of decrease was almost uniform during 0-90 days Paired t test indicate that the d fferences in the bulgaricus count in frozen yogurt between 0 and 90 days were not significant (Table 4 8c) under treatments A-I A-II B-I and B-II The statistically sigrificant reduction in bulgaricus count was recorded in fro en yogurt under A III A-IV B-III and B-IV after the 90 days of storage The count after 90 days of storage under the treatments A-I A-II A-III and A-IV was 0 41 x 10^9 0 45 x 10^9 0 33 x 10^9 and 0 22 x 10^9

Table 4 8a Effect of storage of frozen yogurt at -20°C for 0-90 days on L delbrueckii subsp bulgaricus count under different treatments (Main trial)

Treatments	Period (days) 0	15	30	45	60	75	90
	(cfu per ml x 10^9)						
A-I	1 75 ± 0 96	1 12 ± 0 77	0 77 ± 0 24	0 60 ± 0 15	0 72 ± 0 26	0 26 ± 0 05	0 41 ± 0 16
A-II	1 30 ± 0 76	0 85 ± 0 28	1 05 ± 0 69	0 99 ± 0 15	0 80 ± 0 28	0 38 ± 0 13	0 45 ± 0 14
A-III	2 31 ± 0 75	1 10 ± 0 34	0 96 ± 0 44	1 07 ± 0 20	0 43 ± 0 11	0 77 ± 0 26	0 33 ± 0 04
A IV	1 32 ± 0 31	0 76 ± 0 27	0 45 ± 0 17	0 30 ± 0 06	0 53 ± 0 19	0 95 ± 0 32	0 22 ± 0 04
B-I	1 37 ± 0 93	1 47 ± 0 91	0 72 ± 0 22	0 61 ± 0 26	1 04 ± 0 26	0 27 ± 0 14	0 44 ± 0 18
B-II	1 47 ± 0 53	0 94 ± 0 30	0 56 ± 0 24	1 02 ± 0 16	0 81 ± 0 28	0 26 ± 0 05	0 28 ± 0 02
B-III	2 63 ± 0 73	1 76 ± 0 83	0 72 ± 0 37	0 89 ± 0 24	0 35 ± 0 13	0 99 ± 0 41	0 20 ± 0 05
B IV	1 43 ± 0 36	0 82 ± 0 23	1 03 ± 0 38	0 40 ± 0 11	0 53 ± 0 24	0 90 ± 0 42	0 18 ± 0 04

Each value is the mean of six replications

Table 4 8b Analysis of variance

Period (days)	Source	DF	MS	F
0	Between treatment	7	1 534	0 506 NS
	Within treatment	40	3 031	
15	Between treatment	7	0 727	0 377 NS
	Within treatment	40	1 928	
30	Between treatment	7	0 280	0 317 NS
	Within treatment	40	0 884	
45	Between treatment	7	0 525	2 590 *
	Within treatment	40	0 203	
60	Between treatment	7	0 320	0 991 NS
	Within treatment	40	0 323	
75	Between treatment	7	0 854	2 011 NS
	Within treatment	40	0 425	
90	Between treatment	7	0 076	1 134 NS
	Within treatment	40	0 067	

NS Non-significant * Significant (P<0 05)

Table 4 8c t values

Treatments	0 Vs 15	0 Vs 30	0 Vs 45	0 Vs 60	0 Vs 75	0 Vs 90
A-I	1 40 NS	1 18 NS	1 06 NS	1 03 NS	1 56 NS	1 29 NS
A-II	0 84 NS	0 21 NS	0 34 NS	0 55 NS	1 15 NS	1 02 NS
A-III	1 44 NS	1 31 NS	1 52 NS	2 39 NS	1 72 NS	2 60 *
A-IV	1 13 NS	2 48 NS	3 25 *	1 58 NS	0 72 NS	3 27 *
B-I	0 67 NS	0 73 NS	0 73 NS	0 29 NS	1 11 NS	0 91 NS
B-II	1 20 NS	1 43 NS	0 65 NS	1 01 NS	2 27 NS	2 23 NS
B-III	1 09 NS	2 34 NS	2 24 NS	2 87 *	1 55 NS	3 18 *
B-IV	1 25 NS	0 65 NS	2 49 NS	1 56 NS	0 49 NS	3 15 *

NS Non-significant * - Significant (P<0 05)

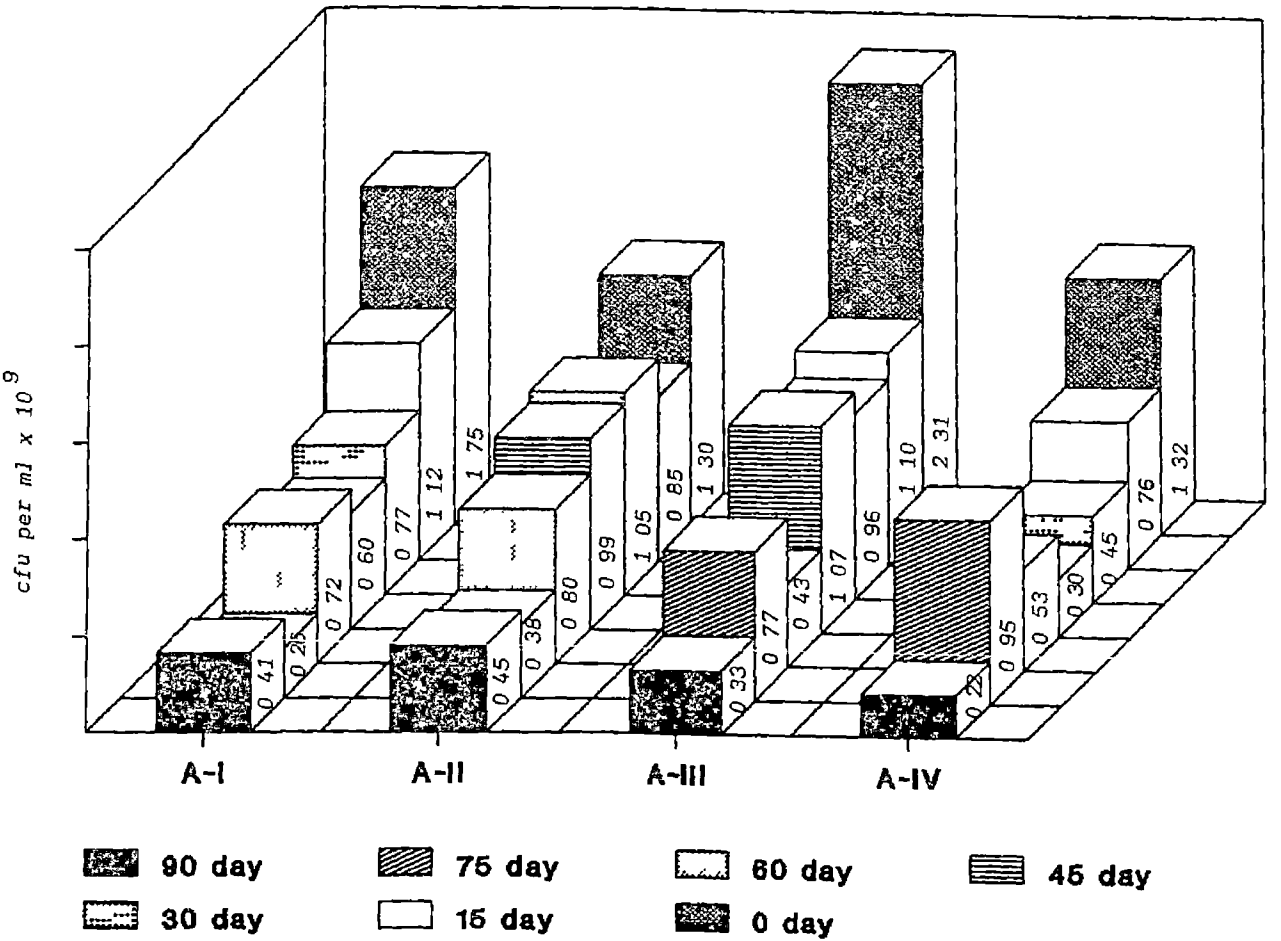


Fig 9a Effect of storage of frozen yogurt on L delbrueckii subsp. bulgricus count under different treatments of part A (Main trial)

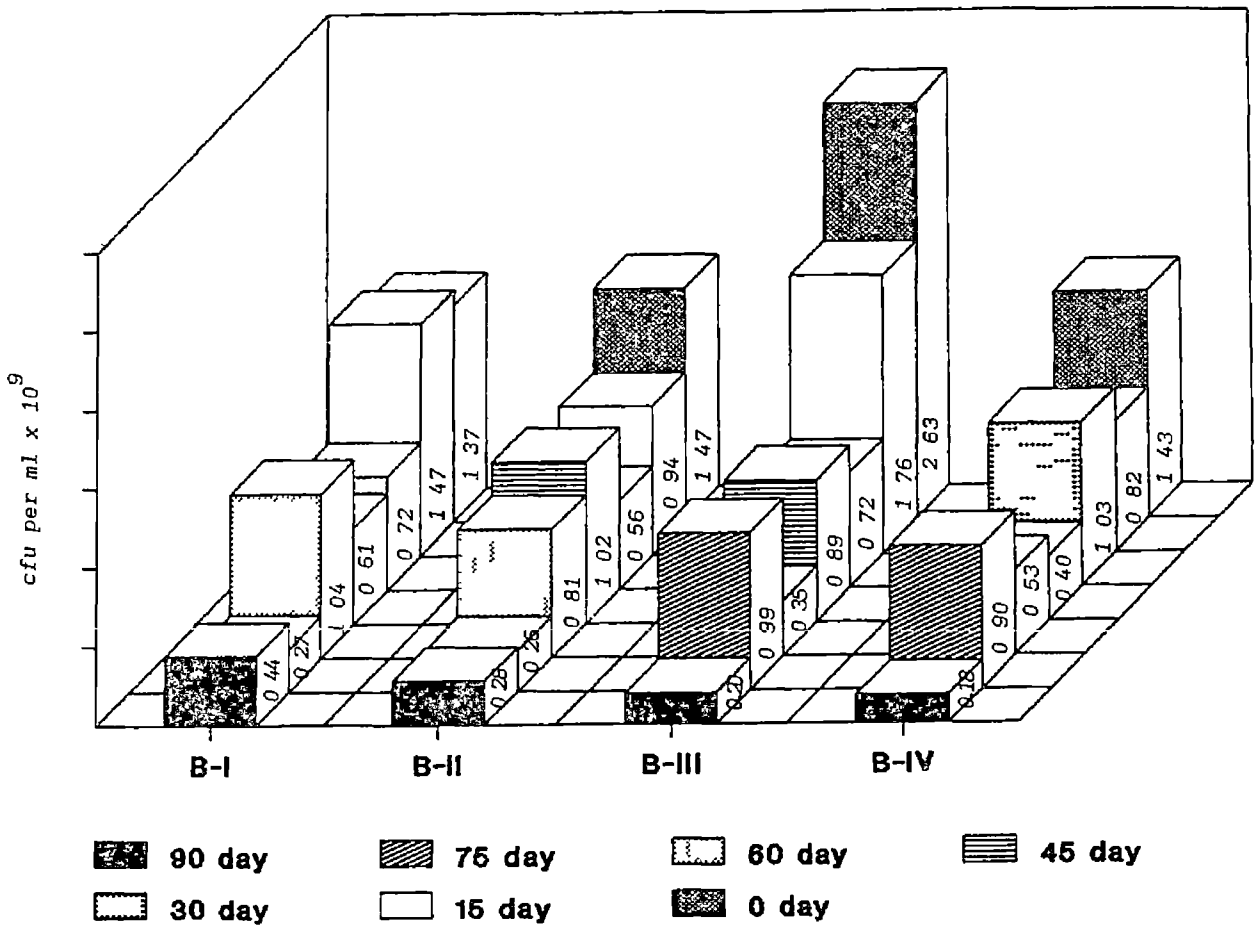


Fig.9b. Effect of storage of frozen yogurt on L delbrueckii subsp. bulgricus count under different treatments of part B (Main trial)

cfu per ml The corresponding values under B-I, B-II, B-III and B-IV were 0 44 x 10^9 0 28 x 10^9, 0 20 x 10^9 and 0 18 x 10^9 cfu per ml respectively

4.9 Effect of storage of frozen yogurt at -20°C for 0-90 days on B bifidum count under different treatments (Main trial)

The bifidobacteria count in frozen yogurt under different treatments gradually decreased during the 90 days of storage (Fig 10) The rate of decrease in the counts were maximum between 0 and 15 days After 15 days the rate of decrease remained steady Paired t test indicate the significant decrease in bifidobacteria population in frozen yogurt between 0 and 15 0 and 30 0 and 45 0 and 60 0 and 75 0 and 90 days of storage under B-II Significant decrease was also recorded between 0 and 45 0 and 75 and 0 and 90 days of storage under B-III and between 0 and 90 days of storage under B-IV No significant difference have been found at different period under B-I (Table 4 9c)

The bifidobacteria count in frozen yogurt after 90 days of storage were 0 13 x 10^9 0 11 x 10^9 0 13 x 10^9 and 0 07 x 10^9 cfu per ml (Table 4 9a) The differences in bifidobacterium count between different treatment in frozen

Table 4 9a Effect of storage of frozen yogurt stored at -20°C for 0-90 days on B bifidum count under different treatments (Main trial)

Treatments	Period (days)						
	0	15	30	45	60	75	90
	(cfu per ml x 10^9)						
B-I	1 54 ± 0 62	0 38 ± 0 02	0 35 ± 0 04	0 35 ± 0 03	0 24 ± 0 02	0 22 ± 0 06	0 13 ± 0 04
B-II	2 63 ± 0 63	0 31 ± 0 04	0 35 ± 0 06	0 30 ± 0 04	0 18 ± 0 06	0 33 ± 0 06	0 11 ± 0 05
B-III	1 85 ± 0 60	0 29 ± 0 02	0 37 ± 0 08	0 25 ± 0 04	0 25 ± 0 07	0 21 ± 0 09	0 13 ± 0 06
B-IV	1 70 ± 0 58	0 32 ± 0 06	0 34 ± 0 03	0 35 ± 0 06	0 31 ± 0 04	0 19 ± 0 08	0 07 ± 0 04

Each value represents the mean of six replications

Table 4 9b Analysis of variance

Period (days)	Source	DF	MS	F
0	Between treatment	3	1 404	0 627 NS
	Within treatment	20	2 241	
15	Between treatment	3	0 011	0 896 NS
	Within treatment	20	0 012	
30	Between treatment	3	0 001	0 047 NS
	Within treatment	20	0 022	
45	Between treatment	3	0 014	0 991 NS
	Within treatment	20	0 015	
60	Between treatment	3	0 017	0 945 NS
	Within treatment	20	0 018	
75	Between treatment	3	0 025	0 661 NS
	Within treatment	20	0 038	
90	Between treatment	3	0 004	0 238 NS
	Within treatment	20	0 018	

NS - Non-significant

Table 4 9c t values

Treatments	0 Vs 15		0 Vs 30		0 Vs 45		0 Vs 60		0 Vs 75		0 Vs 90	
B-I	1 88	NS	1 94	NS	1 95	NS	2 10	NS	2 09	NS	2 28	NS
B-II	3 79	*	3 72	*	3 54	*	3 61	*	3 51	*	3 80	*
B-III	2 55	NS	2 48	NS	2 76	*	2 52	NS	2 65	*	2 92	*
B-IV	2 22	NS	2 30	NS	2 10	NS	2 24	NS	2 37	NS	2 73	*

NS - Non-significant

* - Significant (P<0 05)

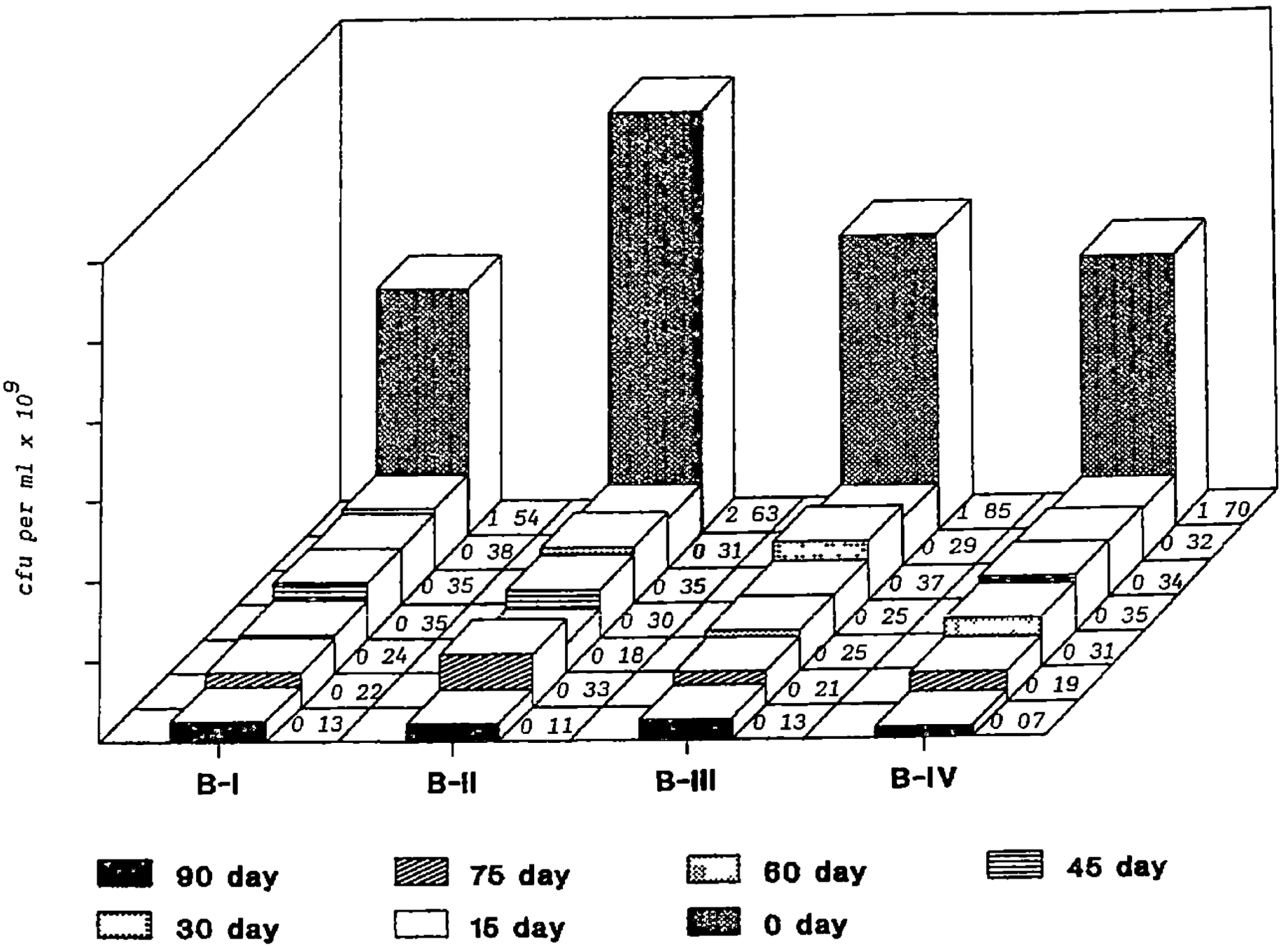


Fig.10. Effect of storage of frozen yogurt on B bifidum count under

yogurt at 0 15 30 45 60 75 and 90 days of storage were found to be statistically not significant (Table 4 9b)

It can be summarised that the counts of thermophilus, bulgaricus and bifidobacteria in frozen yogurt decreased gradually during the storage at -20°C However the overall decrease under different treatment did not exceed more than one log cycle At the end of 90 days of storage sufficient population of these starter bacteria were recorded to have their beneficial effect after consumption

4 10 Effect of storage on viable starter count in frozen yogurt at-20°C for 0-30 days under different treatments (Supporting trial)

The thermophilus bulgaricus and bifidobacteria count in frozen yogurt stored for 0 15 and 30 days under different treatments are presented in Table 4 10a

There was a sharp decrease in the count of all three starter bacteria between 0 and 15 days of storage The decrease in the count between 15 and 30 days were comparatively less (Fig 11 12 and 13)

Highly significant reduction ($P<0.01$) in thermophilus count was recorded between 0 and 15 days (Table 4 10c) of storage of frozen yogurt under all the treatments except

Table 4 10a Effect of storage on viable starter count in frozen yougrt at -20°C for 0-30 days under different treatments (Supporting trial)

Treatments	*S salivarius* subsp *thermophilus*			*L delbrueckii* subsp *bulgaricus*			*B bifidum*		
	Period (days)			Period (days)			Period (days)		
	0	15	30	0	15	30	0	15	30
	(cfu per ml x 10°9°)								
A-I	2 06 ± 0 23	0 36 ± 0 07	0 32 ± 0 03	2 27 ± 0 09	0 36 ± 0 07	0 28 ± 0 02	-	-	-
A III	2 41 ± 0 25	0 78 ± 0 25	0 52 ± 0 12	2 68 ± 0 15	0 73 ± 0 22	0 50 ± 0 14	-	-	-
B-I	2 15 ± 0 18	0 49 ± 0 07	0 45 ± 0 09	1 07 ± 0 05	0 25 ± 0 04	0 25 ± 0 04	2 48 ± 0 24	0 62 ± 0 26	0 60 ± 0 10
B-III	2 43 ± 0 15	1 28 ± 0 48	0 70 ± 0 13	2 55 ± 0 11	1 22 ± 0 45	0 69 ± 0 19	2 32 ± 0 10	0 33 ± 0 04	0 55 ± 0 09
CD				0 47	0 82	0 38			

Each value is the mean of ten replications

Table 4 10b Analysis of variance

Source		DF	MS	F
Count of thermophilus				
0 day	Between treatment	3	0 340	0 766 NS
	Within treatment	36	0 443	
15 day	Between treatment	3	1 675	2 158 NS
	Within treatment	36	0 776	
30 day	Between treatment	3	0 253	2 170 NS
	Within treatment	36	0 116	
Count of bulgaricus				
0 day	Between treatment	3	5 390	49 320 **
	Within treatment	36	0 109	
15 day	Between treatment	3	1 920	2 860 *
	Within treatment	36	0 670	
30 day	Between treatment	3	0 430	2 860 *
	Within treatment	36	0 150	
Count of bifidobacteria				
0 day	Between treatment	1	0 128	0 340 NS
	Within treatment	18	0 350	
15 day	Between treatment	1	0 409	1 160 NS
	Within treatment	18	0 352	
30 day	Between treatment	1	0 013	0 126 NS
	Within treatment	18	0 103	

NS - Non significant * - Significant (P<0 05) ** - Highly significant (P<0 01)

Table 4 10c t values

Treatments	*S salivarius* subsp *thermophilus*		*L delbrueckii* subsp *bulgaricus*		*B bifidum*	
	0 Vs 15	0 Vs 30	0 Vs 15	0 Vs 30	0 Vs 15	0 Vs 30
A-I	7 17 **	7 73 **	12 66 **	17 86 **	-	-
A-III	4 42 **	6 78 **	11 72 **	12 47 **	-	-
B-I	9 49 **	8 72 **	9 57 **	14 81 **	5 75 **	6 30 **
B III	2 13 NS	11 16 **	2 63 *	8 10 **	18 36 **	12 61 **

NS Non-significant * - Significant (P<0 05) ** - Highly significant (P<0 01)

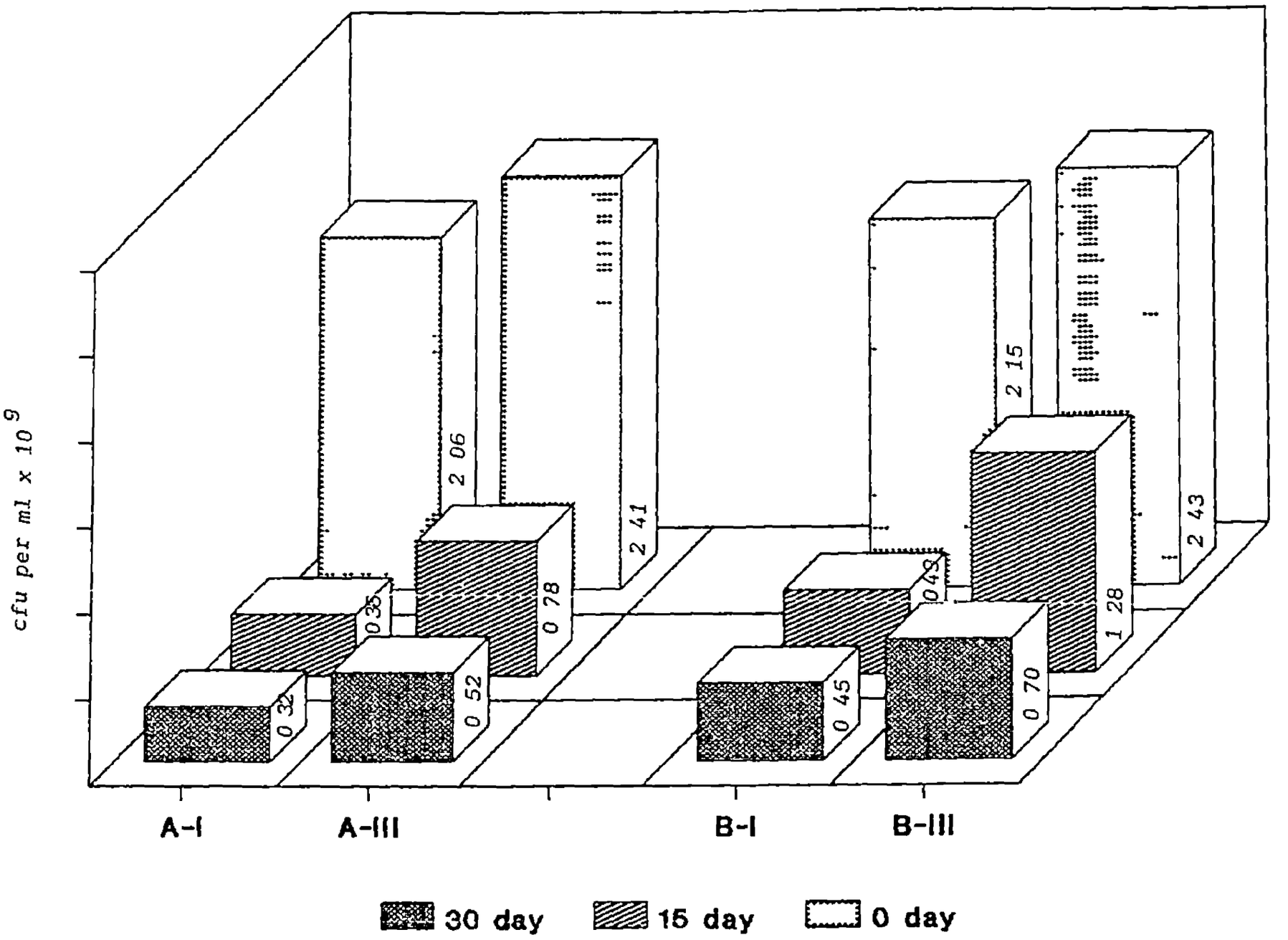


Fig 11 Effect of storage of frozen yogurt on S salivarius subsp

ff. + treatments (Supporting trial)

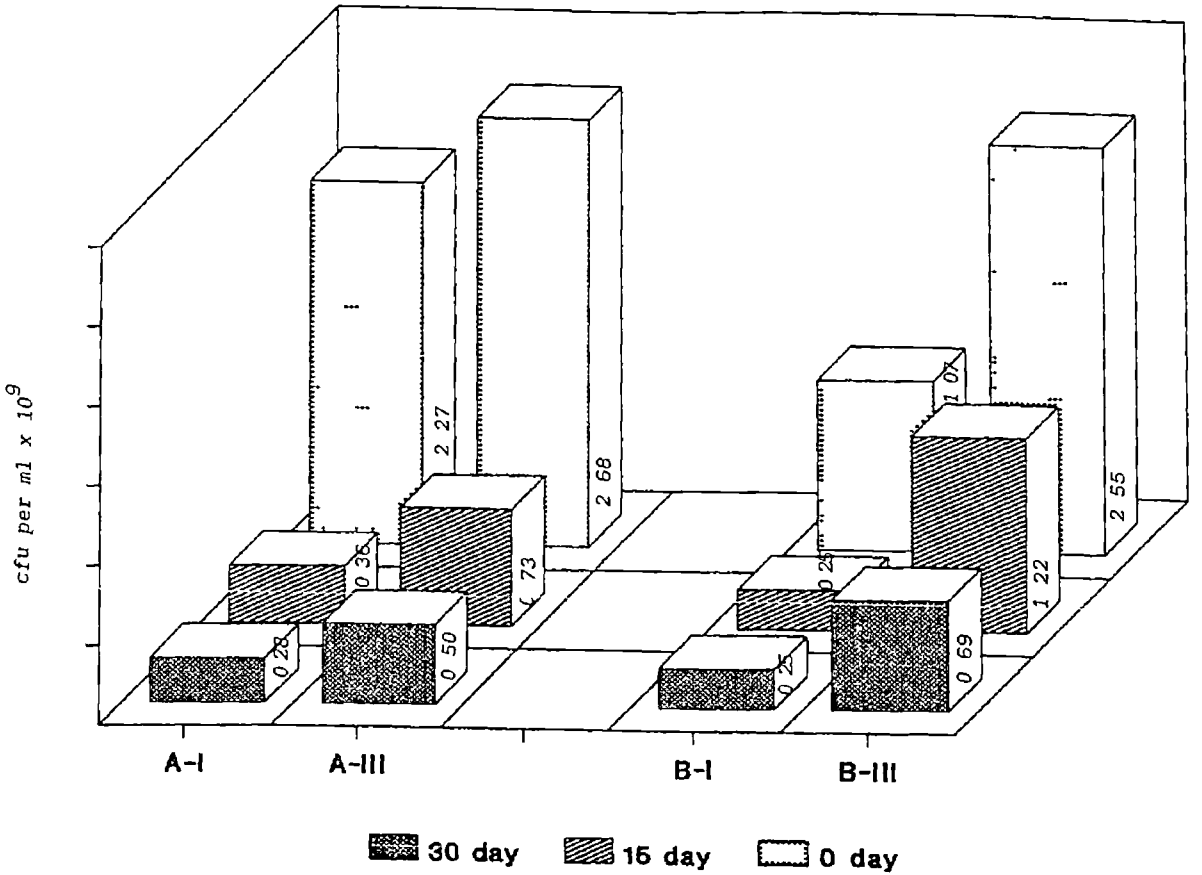


Fig 12 Effect of storage of frozen yogurt on L delbrueckii subsp bulgaricus count under different treatments of (Supporting trial)

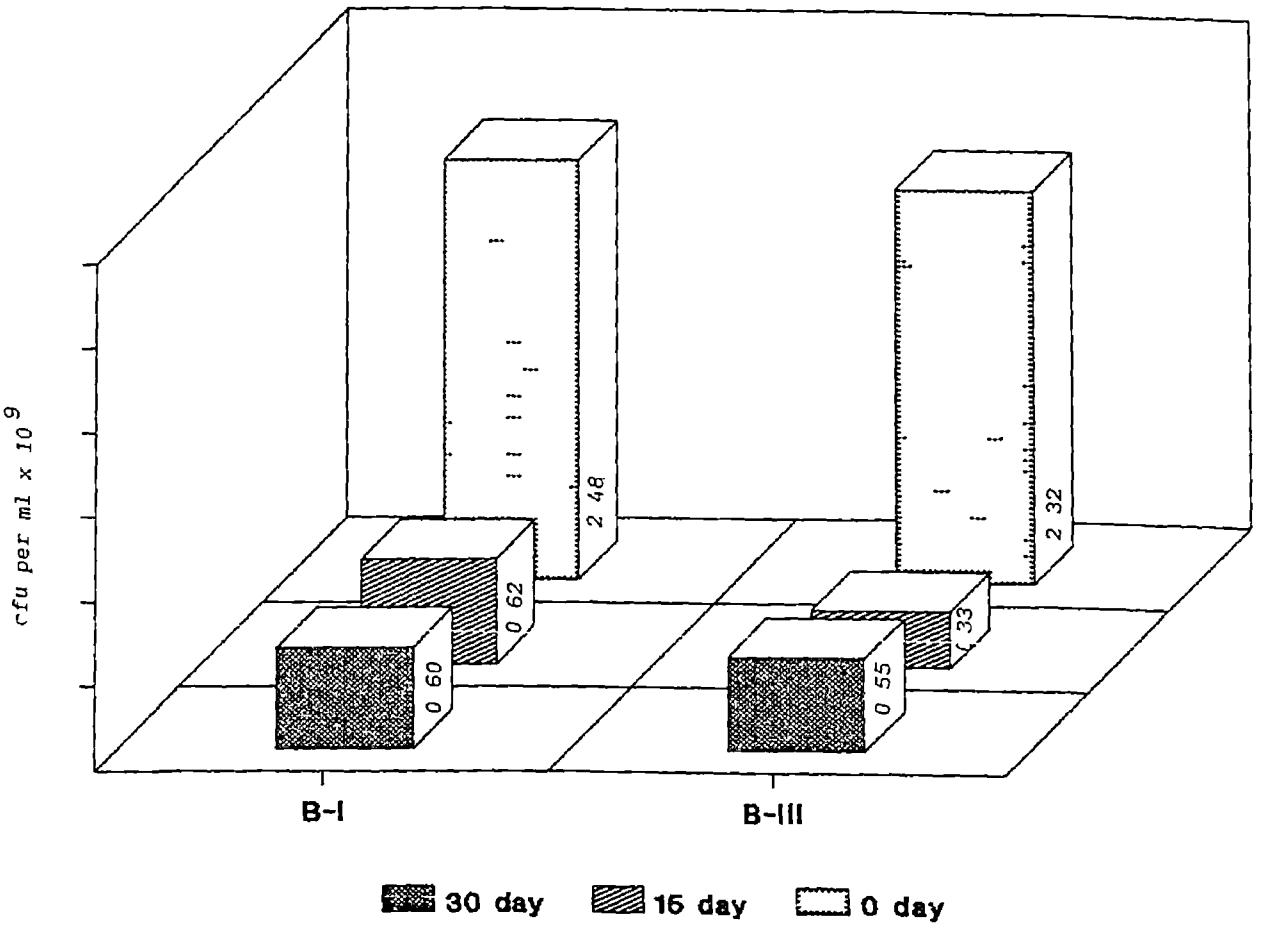


Fig 13 Effect of storage of frozen yogurt on B bifidum count under different treatments (Supporting trial)

B-III The reduction in the thermophilus count was statistically not significant under treatment B-III

Comparison between 0 and 30 days also indicate the highly significant (P<0 01) reduction of the thermophilus count during the storage of frozen yogurt for 30 days

The thermophilus count in frozen yogurt after 15 days of storage was 0 36 x 10^9 0 78 x 10^9 0 49 x 10^9 and 1 28 x 10^9 cfu per ml under treatments A-I A-III B-I and B-III respectively The thermophilus count in frozen yogurt under treatments A-I A-III B-I and B-III were 0 32 x 10^9 0 52 x 10^9 0 45 x 10^9 and 0 70 x 10^9 cfu per ml respectively after 30 days of storage

As regards to bulgaricus and bifidobacteria a highly significant (P<0 01) reduction in the count was recorded for both during storage period of 15 and 30 days (Table 4 10c) under all the treatments

The bulgaricus count at the end of the 30 days storage in frozen yogurt under A-I A III B-I and B-III were 0 28 x 10^9 0 50 x 10^9 0 25 x 10^9 and 0 69 x 10^9 cfu per ml respectively

The bifidobacteria count at the end of 30 days of

storage in frozen yogurt under B-I and B-III were 0 60 x 10^9 and 0 55 x 10^9 cfu per ml respectively

Eventhough a highly significant (P<0 01) reduction in the count of thermophilus bulgaricus and bifidobacteria were recorded under different treatments after the storage of 30 days the extent of reduction was only one to two logarithmic unit

4.11 Coliform, yeast and mould count in set yogurt under different treatments

The coliform yeast and mould count in the samples of set yogurt under different treatments are presented in Table 4 11a

The mean coliform count under treatment A I A-II A-III and A-IV were 1 66 1 66 3 33 and 3 33 cfu per ml respectively The corresponding values under B-I B-II B-III and B IV were 1 66 1 66 3 33 and 5 00 cfu per ml respectively The differences between the counts under different treatments were found to be statistically not significant (Table 4 11b)

The mean values for yeast and mould count in set yogurt under A I A II A-III and A-IV were 3 33 3 33 11 66 and 5 00 cfu per ml respectively The count under B I B II

Table 4 11a Coliform yeast and mould count in set yogurt under different treatments

Treatments	Coliform	Yeast and mould
	(cfu per ml)	
A-I	1 66 ± 1 6	3 33 ± 2 1
A-II	1 66 ± 1 6	3 33 ± 2 1
A-III	3 33 ± 1 9	11 66 ± 6 5
A-IV	3 33 ± 1 2	5 00 ± 3 4
B-I	1 66 ± 1 6	6 67 ± 6 6
B-II	1 66 ± 1 6	3 33 ± 3 3
B-III	3 33 ± 1 9	6 67 ± 4 9
B IV	5 00 ± 4 9	3 33 ± 3 3

Each value is the mean of six replications

Table 4 11b Analysis of variance

Source		D F	MS	F
Coliform count	Between treatment	7	49 70	0 92 NS
	Within treatment	40	53 75	
Yeast and mould count	Between treatment	7	51 19	0 44 NS
	Within treatment	40	115 83	

NS Non significant

B III and B-IV were 6 67 3 33 6 67 and 3 33 cfu per ml respectively The differences in the count between the treatments were found to be not significant (Table 4 11b)

Biochemical analysis

4 12 Comparison of titratable acidity between set and frozen yogurt under different treatments

The titratable acidity expressed as per cent equivalent lactic acid in set yogurt at pH 4 6 and frozen yogurt (0 day) under different treatments are given in Table 4 12a

The mean acidity in set yogurt at pH 4 6 under A-I A-II A-III and A-IV were 0 99 0 93 0 96 and 0 94 per cent equivalent lactic acid The corresponding values under B-I B-II B-III and B-IV were 0 98 0 96 1 02 and 0 98 per cent equivalent lactic acid

The mean acidity in frozen yogurt immediately after freezing and overnight hardening (0 day) under A-I A-II A-III and A-IV were 0 85 0 86 0 37 and 0 93 per cent equivalent lactic acid respectively The corresponding figures under B I B-II B-III and B IV were 0 87 0 86 0 88 and 0 94 per cent equivalent lactic acid respectively

Table 4 12a Comparison of titratable acidity between set and frozen yogurt under different treatments (per cent equivalent lactic acid)

Treatments	Titratable acidity in set yogurt at pH 4 6	Titratable acidity in frozen yogurt	t values
A-I	0 99 ± 0 06	0 85 ± 0 03	1 70 NS
A-II	0 93 ± 0 04	0 86 ± 0 02	1 27 NS
A-III	0 96 ± 0 04	0 87 ± 0 05	1 58 NS
A IV	0 94 ± 0 04	0 93 ± 0 04	0 39 NS
B-I	0 98 ± 0 04	0 87 ± 0 04	2 47 NS
B-II	0 96 ± 0 04	0 86 ± 0 02	2 23 NS
B-III	1 02 ± 0 03	0 88 ± 0 06	2 63 *
B-IV	0 98 ± 0 06	0 94 ± 0 01	0 53 NS

Each value is the mean of six replications

Table 4 12b Analysis of variance

Source		D F	MS	F
Yogurt	Between treatment	7	5 67	0 38 NS
	Within treatment	40	1 48	
Frozen Yogurt	Between treatment	7	0 007	0 84 NS
	Within treatment	40	0 008	

NS - Non-significant * Significant (P<0 05)

Analysis of variance between the treatment indicated no significant differences in acidity in set yogurt and also between the treatments in frozen yogurt (0 day) (Table 4 12b)

Eventhough slight reduction in the acidity during the process of manufacturing of frozen yogurt was observed the reduction was found to be statistically not significant except under B-III (Table 4 12a)

4 13 Titratable acidity in frozen yogurt stored at -20°C for 0-90 days under different treatments

The mean values of titratable acidity expressed as per cent equivalent lactic acid in frozen yogurt stored for 0-90 days are presented in Table 4 13a

The titratable acidity in frozen yogurt under different treatments showed slight increase during the storage period At the end of 90 days of storage the mean acidity under A-I A-II A-III and A IV were 0 91, 0 88 0 92 and 0 85 per cent equivalent lactic acid respectively The corresponding values under B-I B-II B-III and B-IV were 0 92 0 94 0 91 and 0 88 per cent respectively

The differences in the values of acidity between 0 and 90 days were found to be not significant (Table 4 13c)

Table 4 13a Titratable acidity in frozen yogurt stored at -20°C for 0 to 90 days under different treatments (per cent equivalent lactic acid)

Treatments	Period (days)						
	0	15	30	45	60	75	90
A-I	0 85 ± 0 03	0 92 ± 0 04	0 89 ± 0 03	0 94 ± 0 04	0 98 ± 0 03	0 85 ± 0 02	0 91 ± 0 02
A-II	0 86 ± 0 01	0 87 ± 0 03	0 87 ± 0 04	0 90 ± 0 06	0 91 ± 0 05	0 80 ± 0 01	0 88 ± 0 02
A-III	0 87 ± 0 05	0 82 ± 0 02	0 88 ± 0 04	0 99 ± 0 05	0 94 ± 0 04	0 82 ± 0 02	0 92 ± 0 02
A-IV	0 93 ± 0 03	0 88 ± 0 06	0 86 ± 0 03	0 98 ± 0 04	0 97 ± 0 06	0 89 ± 0 03	0 85 ± 0 01
B I	0 87 ± 0 03	0 93 ± 0 02	0 93 ± 0 03	1 04 ± 0 04	1 02 ± 0 03	0 90 ± 0 03	0 92 ± 0 03
B-II	0 86 ± 0 01	0 88 ± 0 02	0 89 ± 0 03	1 02 ± 0 06	0 92 ± 0 02	0 88 ± 0 03	0 94 ± 0 02
B-III	0 88 ± 0 05	0 86 ± 0 02	0 86 ± 0 04	1 03 ± 0 04	0 94 ± 0 03	0 85 ± 0 02	0 91 ± 0 01
B IV	0 94 ± 0 01	0 90 ± 0 06	0 90 ± 0 03	0 98 ± 0 04	1 01 ± 0 04	0 90 ± 0 03	0 88 ± 0 02

Each value is the mean of six replications

4 13b Analysis of variance

Period (days)	Source	DF	MS	F
0	Between treatment	7	0 007	0 84 NS
	Within treatment	40	0 008	
15	Between treatment	7	0 007	0 82 NS
	Within treatment	40	0 009	
30	Between treatment	7	0 003	0 42 NS
	Within treatment	40	0 008	
45	Between treatment	7	0 004	0 24 NS
	Within treatment	40	0 016	
60	Between treatment	7	0 010	0 81 NS
	Within treatment	40	0 012	
75	Between treatment	7	0 008	1 51 NS
	Within treatment	40	0 005	
90	Between treatment	7	0 005	1 54 NS
	Within treatment	40	0 004	

NS - Non-significant

Table 4.13c. t values

Treatments	0 Vs 15	0 Vs 30	0 Vs 45	0 Vs 60	0 Vs 75	0 Vs 90
A-1	1.97 NS	1.24 NS	6.03 **	4.69 **	0.18 NS	1.44 NS
A-II	0.58 NS	0.23 NS	2.75 *	3.92 NS	1.87 NS	1.61 NS
A-III	1.12 NS	0.41 NS	2.10 NS	2.47 NS	0.75 NS	1.03 NS
A-IV	0.58 NS	1.22 NS	1.02 NS	0.56 NS	0.56 NS	1.78 NS
B-I	1.88 NS	1.29 NS	5.12 **	4.36 **	0.44 NS	1.21 NS
B-II	1.07 NS	1.21 NS	3.84 *	2.71 *	0.54 NS	2.35 NS
B-III	0.23 NS	0.58 NS	2.43 NS	1.03 NS	0.53 NS	0.78 NS
B-IV	0.73 NS	1.13 NS	1.24 NS	2.10 NS	0.81 NS	1.65 NS

NS - Non-significant * - Significant (P<0.05) ** - Highly significant (P<0.01)

4 14 pH of frozen yogurt stored at -20°C during 0-90 days under different treatments

The pH of frozen yogurt after freezing and overnight hardening (0 day) and stored for 90 days at -20°C under different treatments are presented in Table 4 14a

The mean values of pH of frozen yogurt (0 day) under A-I A-II A III and A-IV were 4 84 4 74 4 77 and 4 69 respectively The corresponding values under B-I, B-II, B III and B IV were 4 74 4 72, 4 73 and 4 71 respectively The differences between treatments were found to be statistically not significant (Table 4 14b)

There were slight apparent decrease in pH of frozen yogurt during the storage period On 90 day the pH under A-I A-II A-III and A-IV were 4 70 4 68 4 66 and 4 60 The corresponding values under B-I B-II B-III and B-IV were 4 65, 4 64 4 63 and 4 57 respectively The comparison of pH values between 0 and 90 days however indicate that the decrease in pH was statistically not significant for all the treatments except under A-I (Table 4 14c)

A slight but statistically significant reduction in pH was observed in frozen yogurt under A-I

Table 4 14a pH of frozen yogurt stored at -20°C for 0 to 90 days under different treatments

Treatments	Period (days)						
	0	15	30	45	60	75	90
A-I	4 84 ± 0 03	4 71 ± 0 04	4 62 ± 0 01	4 65 ± 0 02	4 76 ± 0 04	4 61 ± 0 001	4 70 ± 0 01
A-II	4 74 ± 0 08	4 70 ± 0 07	4 68 ± 0 03	4 65 ± 0 02	4 74 ± 0 02	4 70 ± 0 001	4 68 ± 0 01
A-III	4 77 ± 0 08	4 72 ± 0 05	4 72 ± 0 04	4 65 ± 0 02	4 64 ± 0 02	4 67 ± 0 02	4 66 ± 0 04
A-IV	4 69 ± 0 10	4 80 ± 0 07	4 70 ± 0 03	4 66 ± 0 02	4 64 ± 0 02	4 62 ± 0 01	4 60 ± 0 01
B-I	4 74 ± 0 03	4 68 ± 0 06	4 61 ± 0 01	4 63 ± 0 02	4 74 ± 0 04	4 61 ± 0 001	4 65 ± 0 02
B-II	4 72 ± 0 04	4 68 ± 0 05	4 67 ± 0 01	4 68 ± 0 03	4 73 ± 0 02	4 69 ± 0 02	4 64 ± 0 02
B-III	4 73 ± 0 06	4 66 ± 0 07	4 68 ± 0 03	4 66 ± 0 02	4 64 ± 0 02	4 66 ± 0 02	4 63 ± 0 02
B-IV	4 71 ± 0 08	4 68 ± 0 09	4 65 ± 0 03	4 63 ± 0 02	4 62 ± 0 01	4 58 ± 0 01	4 57 ± 0 01
CD					0 10	0 07	0 09

Each value is the mean of six replications

Table 4.14b. Analysis of variance

Period (days)	Source	DF	MS	F
0	Between treatment	7	0.013	0.42 NS
	Within treatment	40	0.033	
15	Between treatment	7	0.012	0.44 NS
	Within treatment	40	0.027	
30	Between treatment	7	0.008	1.53 NS
	Within treatment	40	0.005	
45	Between treatment	7	0.002	0.63 NS
	Within treatment	40	0.003	
60	Between treatment	7	0.021	4.36 **
	Within treatment	4 0	0.005	
75	Between treatment	7	0.012	8.35 **
	Within treatment	40	0.001	
90	Between traafmpnt-	7	Q ,Q1Q	2.71 *
	Within treatment	40	0.004	

NS - Non-significant * - Significant (P<0.05) ** - Highly significant (P<0.01)

Table 4 14c t values

Treatments	0 Vs 15	0 Vs 30	0 Vs 45	0 Vs 60	0 Vs 75	0 Vs 90
A I	3 74 *	7 65 **	6 68 **	1 55 NS	6 17 **	3 17 *
A-II	0 76 NS	0 95 NS	1 21 NS	0 02 NS	0 48 NS	0 63 NS
A-III	0 85 NS	0 72 NS	1 66 NS	1 56 NS	1 26 NS	0 95 NS
A-IV	0 81 NS	0 72 NS	0 53 NS	0 43 NS	0 68 NS	0 86 NS
B-I	1 77 NS	3 68 *	2 19 NS	0 12 NS	2 89 *	1 93 NS
B II	0 76 NS	1 26 NS	0 86 NS	0 23 NS	0 41 NS	1 23 NS
B-III	1 88 NS	0 82 NS	0 96 NS	1 14 NS	1 07 NS	1 23 NS
B IV	0 53 NS	0 59 NS	0 79 NS	0 85 NS	1 27 NS	1 48 NS

NS - Non-significant * Significant (P<0 05) ** - Highly significant (P<0 01)

4 15 Contents of total solids in set yogurt under different treatments

The total solids percentage in set yogurt under different treatments are presented in Table 4 15a

Total solids percentage in set yogurt under A-I A-II A-III and A-IV were 21 32 21 02 21 73 and 20 86 respectively The corresponding values under B-I B-II B-III and B-IV were 21 52 21 55 21 65 and 21 33 respectively The differences between the treatments were found to be statistically not significant (Table 4 15b)

4 16 Content of Non Protein Nitrogen (NPN) in set yogurt under different treatments

The content of NPN in yogurt under A-I A-II, A-III and A-IV were 63 03 52 53, 46 69 and 60 69 mg per 100 g respectively (Table 4 16a) The values under A-I and A-IV were very close and higher than A-II and A-III The corresponding values under B-I B-II B-III and B-IV were 51 36 58 36 49 61 and 63 03 mg per 100 g respectively Perusal of the values of NPN under treatments of part A and part B revealed great variation within part A and B No set pattern between values was observed The analysis of variance (Table 4 16b) however revealed no significant differences between the treatments To interpret the values of NPN the

Table 4 15a Content of total solids in set yogurt under different treatments

Treatments	Total solids (per cent)
A-I	21 32 ± 0 10
A II	21 02 ± 0 24
A-III	21 73 ± 0 42
A IV	20 86 ± 0 15
B-I	21 52 ± 0 17
B-II	21 55 ± 0 27
B-III	21 65 ± 0 20
B-IV	21 33 ± 0 13

Each value represents the mean of six replications

Table 4 15b Analysis of variance

Source	D f	MS	F
Between treatment	7	0 559	0 65 NS
Within treatment	40	0 340	

NS - Non-significant

Table 4 16a Content of non protein nitrogen (NPN) in set yogurt under different treatments (ml per 100 g)

Treatments	A	B	Combined mean of part A & B
	(1)	(2)	(3)
I	63 03 ± 9 56	51 36 ± 8 76	57 20
II	52 53 ± 11 58	58 36 ± 13 24	55 45
III	46 69 ± 9 80	49 61 ± 8 78	48 15
IV	60 69 ± 8 97	63 03 ± 10 77	61 86
Combined means of I II III&IV	55 74	55 59	

Each value in column (1) and (2) represent the mean of six replication

Each value in the column (3) was obtained from the combination of six replication from part A and six replication from part B Thus each value represent the mean of 12 replication

Table 4 16b Analysis of variance

Source	DF	MS	F
Between treatment	7	245 796	0 38 NS
Within treatment	40	645 055	

NS Not significant

data were regrouped under I II III and IV by combining the values from part A and part B The overall means were calculated based on 12 observation (6 each from A and B) and data was analysed as per the method of Mead (1988)

The overall mean for treatments under part A and B were 55 74 and 55 59 respectively There was no statistically significant difference between two

The overall mean values for NPN under I, II III and IV was 57 20 55 45 48 15 and 61 86 mg per 100 g respectively (Table 4 16a)

The analysis of variance revealed the significant difference between the treatments The calculated CD was 9 65 The NPN content in yogurt under IV was significantly higher ($P<0$ 05) than that of III and at par with I and II

4 17 Comparison of tyrosine values in set and frozen yogurt under different treatments

The tyrosine value in set and frozen yogurt after freezing and hardening (0 day) under different treatments are given in (Table 4 17a)

Tyrosine value in set yogurt under treatments A-I, A-II A-III and A IV were 0 18 0 20, 0 17 and 0 24 mg per g respectively The corresponding values under treatment B-I

Table 4 17a Comparison of tyrosine values in set and frozen yogurt under different treatments

Treatments	Mean tyrosine value mg/g		t values
	Set yogurt	Frozen yogurt	
A-I	0 18 ± 0 03	0 17 ± 0 04	0 35 NS
A-II	0 20 ± 0 02	0 17 ± 0 03	2 75 *
A-III	0 17 ± 0 03	0 22 ± 0 02	1 25 NS
A IV	0 24 ± 0 01	0 25 ± 0 02	0 33 NS
B-I	0.20 ± 0 01	0 19 ± 0 03	0 40 NS
B II	0 21 ± 0 02	0 19 ± 0 03	1 13 NS
B-III	0 19 ± 0 02	0 23 ± 0 01	0 91 NS
B-IV	0 24 ± 0 01	0 26 ± 0 001	1 22 NS

Each value is the mean of six replications

NS - Non-significant * Significant (P<0 05)

Table 4 17b Analysis of variance

Source		DF	MS	F
Set yogurt	Between treatment	7	0 004	1 25 NS
	Within treatment	40	0 003	
Frozen Yogurt	Between treatment	7	0 008	1 91 NS
	Within treatment	40	0 004	

NS - Non significant

B-II B-III and B IV were 0 20 0 21 0 19 and 0 24 respectively

The tyrosine value under treatments of part A were lower than corresponding values under part B The values under A-IV and B-IV were exactly same The tyrosine value under treatment IV (A IV and B IV) was substantially higher than treatment I (A-I and B-I) The differences in the treatment were found to be not significant (Table 4 17b)

The tyrosine value in frozen yogurt (0 day) under A-I A-II A III and A IV were 0 17 0 17 0 22 and 0 25 mg per g respectively The corresponding figure under B-I B-II B-III and B-IV were 0 19 0 19 0 23 and 0 25 mg per g respectively

The perusal of the figures in Table 4 17a indicate that the tyrosine value of frozen yogurt (0 day) under A-II was marginally but significantly lower than that of set yogurt

The tyrosine value of frozen yogurt (0 day) under the treatment III and IV (in both part A and B) were higher than that of set yogurt The increase in the value was by 0 04-0 05 mg per g

4 18 Tyrosine values in frozen yogurt stored at -20°C for 0-90 days, under different treatments

The tyrosine values in frozen yogurt stored for 0-90 days and analysed at 15 days interval were presented Table 4 18a

It was observed that there was an increase in tyrosine values of frozen yogurt under all the treatments with the progress in storage period The increase was abrupt between 0 and 15 days of storage under A I A-II B-I and B-II thereafter the values increased steadily The increase in tyrosine value under A III A-IV B-III and B-IV was slow from the beginning of storage period The tyrosine value in frozen yogurt on 90 days of storage under A-I A-II, A-III and A-IV were 0 27 0 27 0 28 and 0 27 mg per g respectively The corresponding values under B-I B-II B-III and B-IV were 0 27 0 27 0 27 and 0 28 mg per g respectively The differences in tyrosine value between 0 and 90 day were statistically significant under A-II, B II and B-III and in other treatment it was found to be not significant (Table 4 18c)

Table 4 18a Tyrosine values in frozen yogurt stored at -20°C for 0-90 days under different treatments

Treatments	Period (days)						
	0	15	30	45	60	75	90
	(mg per g)						
A I	0 17 ± 0 04	0 25 ± 0 01	0 25 ± 0 01	0 27 ± 0 01	0 27 ± 0 01	0 26 ± 0 01	0 27 ± 0 01
A-II	0 17 ± 0 03	0 23 ± 0 01	0 24 ± 0 01	0 25 ± 0 01	0 26 ± 0 01	0 27 ± 0 01	0 27 ± 0 01
A-III	0 22 ± 0 02	0 23 ± 0 01	0 24 ± 0 01	0 25 ± 0 01	0 26 ± 0 01	0 27 ± 0 01	0 28 ± 0 01
A-IV	0 25 ± 0 02	0 21 ± 0 01	0 24 ± 0 02	0 26 ± 0 01	0 27 ± 0 01	0 27 ± 0 01	0 27 ± 0 01
B I	0 19 ± 0 03	0 24 ± 0 01	0 24 ± 0 02	0 27 ± 0 01	0 27 ± 0 01	0 27 ± 0 01	0 27 ± 0 01
B-II	0 19 ± 0 03	0 22 ± 0 01	0 23 ± 0 02	0 26 ± 0 01	0 26 ± 0 01	0 27 ± 0 01	0 27 ± 0 01
B-III	0 23 ± 0 01	0 23 ± 0 02	0 23 ± 0 02	0 26 ± 0 01	0 26 ± 0 01	0 26 ± 0 01	0 27 ± 0 01
B-IV	0 26 ± 0 01	0 22 ± 0 01	0 24 ± 0 01	0 27 ± 0 01	0 27 ± 0 01	0 27 ± 0 01	0 28 ± 0 01

Each value is the mean of six replications

Table 4 18b Analysis of variance

Period (days)	Source	DF	MS	F
0	Between treatment	7	0 00800	1 91 NS
	Within treatment	40	0 00400	
15	Between treatment	7	0 00110	1 18 NS
	Within treatment	40	0 00100	
30	Between treatment	7	0 00030	0 36 NS
	Within treatment	40	0 00100	
45	Between treatment	7	0 00013	1 29 NS
	Within treatment	40	0 00010	
60	Between treatment	7	0 00027	2 72 *
	Within treatment	40	0 00010	
75	Between treatment	7	0 00082	0 82 NS
	Within treatment	40	0 00100	
90	Between treatment	7	0 06400	0 64 NS
	Within treatment	40	0 10000	

NS - Non-significant

Table 4.18c. t values

Treatments	0 Vs 15	0 Vs 30	0 Vs 45	0 Vs 60	0 Vs 75	0 Vs 90
A-1	1.96 NS	1.98 NS	1.90 NS	2.28 NS	2.37 NS	2.51 NS
A-II	2.09 NS	2.03 NS	2.72 *	2.89 *	3.25 *	3.31 *
A-III	0.59 NS	1.00 NS	2.05 NS	2.43 NS	3.55 *	0.00 NS
A-IV	2.17 NS	0.46 NS	0.85 NS	1.49 NS	1.51 NS	**1.49** NS
B-I	1.17 NS	1.15 NS	2.10 NS	2.12 NS	2.19 NS	2.39 NS
B-II	1.08 NS	0.94 NS	2.12 NS	2.30 NS	2.68 *	2.79 *
B-III	0.41 NS	0.41 NS	0.00 NS	2.35 NS	2.61 *	3.16 *
B-IV	3.41 *	1.88 NS	0.92 NS	0.48 NS	0.86 NS	0.00 NS

NS - Non-significant * - Significant (P<0.05)

4 19 Contents of diacetyl and acetaldehyde in set yogurt under different treatments

The content of diacetyl acetaldehyde and their ratio in set yogurt are given in Table 4 19a

The diacetyl content in set yogurt under treatments A-I A-II A-III and A-IV was 13 92 12 80 10 00 and 19 00 ppm respectively The corresponding figures under B-I B-II B III and B IV were 10 17 10 13, 7 83 and 15 17 ppm respectively

The diacetyl content under the treatments of part B were found to be lower than the corresponding treatment of part A (Fig 14) The diacetyl values under treatment IV (both in A-IV and B IV) were substantially higher than that of treatment I (A-I and B-I) The content of diacetyl under treatments of II and III (both in A and B part) were lesser than their respective control (A-I and B-I)

These differences were found to be statistically not significant (Table 4 19b)

The acetaldehyde content under A-I A-II A III and A-IV were 36 50 33 67 43 04 and 31 50 ppm respectively (Fig 14) The corresponding values under B-I B-II B-III and B-IV were 31 88 34 46 34 52 and 32 42 ppm respectively The

Table 4 19a Contents of diacetyl and acetaldehyde in set yogurt under different treatments

Treatments	Diacetyl (ppm)	Acetaldehyde (ppm)	Diacetyl-acetaldehyde ratio
A-I	13 92 ± 2 82	36 50 ± 1 32	2 62 ± 0 20
A II	12 80 ± 3 64	33 67 ± 0 08	2 63 ± 0 83
A-III	10 00 ± 1 63	43 04 ± 0 99	4 30 ± 3 19
A-IV	19 00 ± 4 44	31 50 ± 0 15	1 66 ± 1 49
B-I	10 17 ± 1 30	31 88 ± 2 65	3 14 ± 0 79
B-II	10 13 ± 0 93	34 46 ± 2 14	3 40 ± 0 48
B-III	7 83 ± 1 08	34 52 ± 4 81	4 41 ± 1 81
B-IV	15 17 ± 3 22	32 42 ± 2 04	2 14 ± 0 44
CD		8 03	

Each value represents the mean of six replications

Table 4 19b Analysis of variance

	Source	DF	MS	F
Diacetyl	Between treatment	7	77 494	1 76 NS
	Within treatment	40	43 922	
Acetaldehyde	Between treatment	7	83 486	2 58 *
	Within treatment	40	32 339	
Diacetyl-acetaldehyde ratio	Between treatment	7	12 549	0 95 NS
	Within treatment	40	13 192	

NS - Non-significant * - Significant (P<0 05)

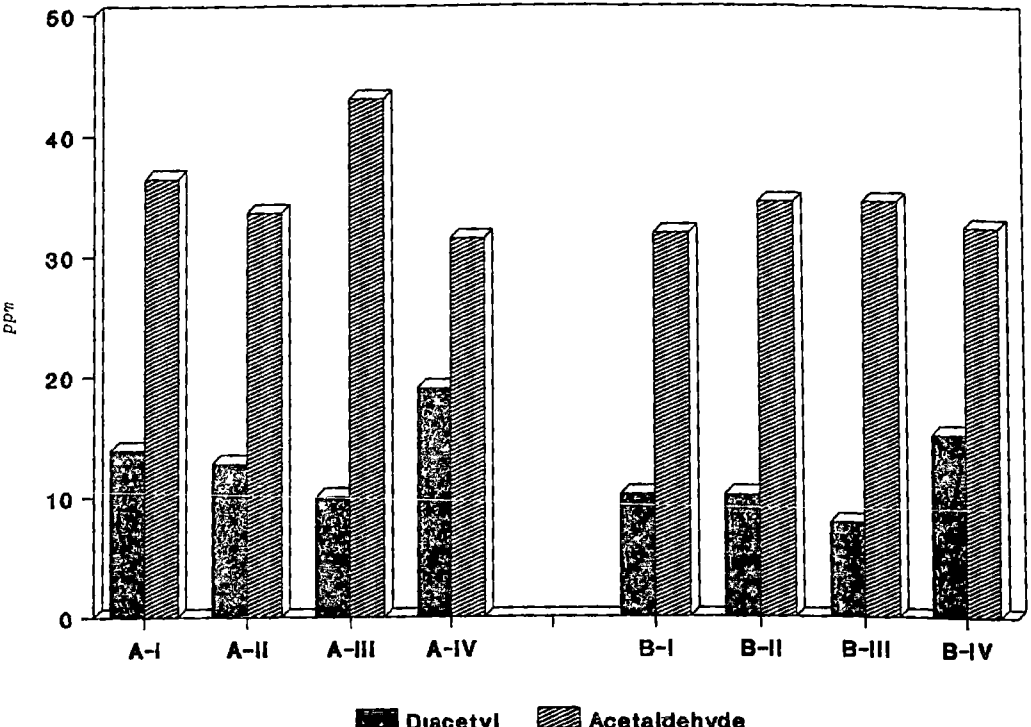


Fig 14. Content of diacetyl and acetaldehyde in set yogurt under different treatments

acetaldehyde content under A-III was significantly (P<0 01) higher than A-II A-IV and B-III and at par with A-I

The diacetyl-acetaldehyde ratio under A-I A-II A-III and A-IV were 2 62 2 63 4 30 and 1 66 respectively The corresponding ratio under B-I B II B III and B-IV were 3 14 3 40 4 41 and 2 14 respectively The ratio under treatment III (both A-III and B-III) was very high while in A-IV was very low The differences in the ratio between the treatment were not statistically significant (Table 4 19b)

4 20 Contents of acetic acid in set and frozen yogurt stored at -20°C for 0, 45 and 90 days under different treatments

The content of acetic acid in set and frozen yogurt are presented in Table 4 20

The acetic acid content unaer treatment A-I A-II A III and A IV were not detected by the gas chromatograph (Chromosorb 101) The average values for acetic acid in set yogurt under B-I B II B-III and B-IV were 0 21 0 14 0 16 and 0 08 per cent respectively

The content of acetic acid in set yogurt fortified with NDM (B I) was substantially higher than that of yogurt

Table 4 20 Contents of acetic acid in set and frozen yogurt stored at -20°C for 0 45 and 90 days under different treatments

Treatments	Yogurt (per cent)	Frozen yogurt (per cent)		
		0	45	90
A-I	Not detectable	Not detectable	Not detectable	Not detectable
A-II	-do-	-do-	-do-	-do-
A III	-do-	-do-	do-	-do-
A IV	-do	-do-	-do-	-do-
B-I	0 21	0 22	0 20	0 21
B-II	0 14	0 15	0 11	0 09
B III	0 16	0 16	0 12	0 15
B-IV	0 08	0 09	0 09	0 06

Each value is the mean of two replications

Table 4 21 Lactic acid acetic acid ratio in set yogurt under different treatments

Treatments	Lactic acid (per cent)	Acetic acid (per cent)	Ratio
B-I	0 77	0 21	0 27
B-II	0 82	0 14	0 17
B-III	0 86	0 16	0 19
B-IV	0 90	0 08	0 09

fortified with condensed whey (B-II and B-III) and whey protein dispersion (B IV)

The values of acetic acid in frozen yogurt (0 day) were 0 22 0 15 0 16 and 0 09 per cent under B-I, B-II, B-III and B-IV respectively A very slight differences in values of acetic acid between set and frozen yogurt (0 day) was observed

After 90 days of storage the values under B-II and B-IV were reduced to 0 09 and 0 06 per cent respectively The values under B-I and B-III remain more or less unchanged during the storage

4.21 Lactic acid - acetic acid ratio in set yogurt under different treatments

The titratable acidity for yogurt recorded under the treatments of part B (yogurt prepared from the combination of thermophilus bulgaricus and bifidobacteria) includes the lactic acid and acetic acid as the bifidobacteria are reported to produce a substantial amount of acetic acid along with lactic acid during glucose metabolism To know the quantity of lactic acid produced in yogurt under part B the average values of acetic acid were subtracted from the average values of titratable acidity in corresponding treatments The calculated values of lactic acid were used for obtaining the

ratio between lactic and acetic acid The ratio under different treatment are presented in Table 4 21 The lactic acid-acetic acid ratio in yogurt under B-I B-II B-III and B IV were 0 27 0 17 0 19 and 0 09 respectively

Sensory evaluation

4 22 Score for the organoleptic characters of set yogurt under different treatments

The mean organoleptic scores of set yogurt for general appearance body and texture flavour and total score are given in Table 4 22a and depicted in Fig 15

The mean score for general appearance under A I A-II A-III and A-IV were 4 28 3 74 4 27 and 4 46 respectively Corresponding values under B-I B-II B-III and B-IV were 3 96 3 84 3 84 and 4 25 respectively The general appearance score under treatment IV (both A-IV and B-IV) was higher than I (A-I and B I) The general appearance under A-I A-III and A-IV were slightly higher than corresponding treatment under part B

The differences between the treatments were found to be statistically not significant (Table 4 22b)

The body and texture score in set yogurt under A I A-II A III and A-IV were 4 18 3 90 3 98 and 4 50 The

Table 4 22a Score for organoleptic characters of set yogurt under different treatments

Treatments	General appearance	Body and texture	Flavour	Total
A-I	4 28 ± 0 11	4 18 ± 0 14	8 75 ± 0 12	17 23 ± 0 21
A-II	3 74 ± 0 21	3 90 ± 0 15	8 43 ± 0 19	16 11 ± 0 42
A-III	4 27 ± 0 06	3 98 ± 0 11	8 55 ± 0 27	16 36 ± 0 37
A-IV	4 46 ± 0 19	4 50 ± 0 05	8 81 ± 0 15	17 80 ± 0 29
B-I	3 96 ± 0 14	4 05 ± 0 17	8 07 ± 0 25	16 17 ± 0 39
B-II	3 84 ± 0 21	3 83 ± 0 13	7 72 ± 0 46	15 55 ± 0 71
B-III	3 84 ± 0 22	3 95 ± 0 12	7 87 ± 0 46	15 38 ± 0 43
B-IV	4 25 ± 0 15	4 20 ± 0 11	8 22 ± 0 20	16 67 ± 0 27
CD		0 39		1 19

Each value is the mean of six replications

Table 4 22b Analysis of variance

Characteristics	Source	DF	MS	F
General appearance	Between treatment	7	0 423	2 110 NS
	Within treatment	40	0 200	
Body and texture	Between treatment	7	0 276	2 518 *
	Within treatment	40	0 109	
Flavour	Between treatment	7	0 976	1 730 NS
	Within treatment	40	0 564	
Total	Between treatment	7	3 957	3 834 **
	Within treatment	40	1 032	

NS Non significant * - Significant (P<0 05)

** - Highly significant (P<0 01)

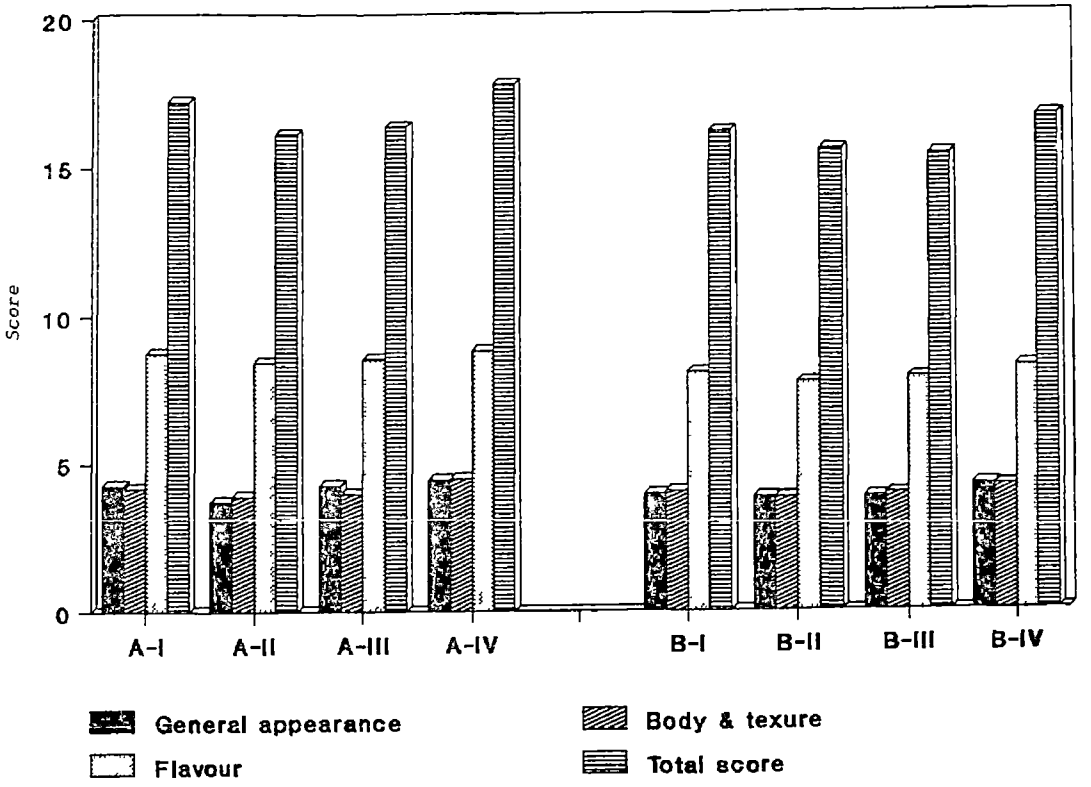


Fig 15 Scores for organoleptic characteristics of set yogurt under different treatments

corresponding values under B-I B II B-III and B-IV were 4 05 3 83 3 95 and 4 20 respectively The body and texture score under A IV was significantly higher than A II and A-III The score under A I and A-IV however were at par with each other The differences in the body and texture score under the treatments of part B were statistically not significant (Table 4 22b) The body and texture score under the treatments of part B were slightly lower than the corresponding treatment of part A

The flavour scores under A-I A-II A-III and A-IV were 8 75 8 43 8 55 and 8 81 respectively The corresponding value under B-I B II B-III and B IV were 8 07 7 72 7 87 and 8 22 respectively The flavour score under A-IV was highest and very close to control (A-I) The scores under A-II and A-III were substantially lower than A-I The same trend was noticed under the treatments of part B The comparison of A and B indicate that the flavour scores under all the treatment of part B were lower than the corresponding treatments of part A The differences in flavour score under different treatments were found to be statistically not significant (Table 4 22b)

Total score under treatment A-I A-II A-III A-IV were 17 23 16 11 16 36 and 17 80 respectively The corresponding values under B-I B-II B-III and B-IV were

16 17 15 55 15 38 and 16 67 respectively The total score under A IV was highest and close to A-I The total score under A-II and A-III were significantly lower than A-IV The total score under part B were comparatively lower than the corresponding treatment of part A Comparison among the treatments of part B indicate that the total score under B IV was significantly higher than B III and at par with B-I and B-II (Table 4 22b)

4 23 Score for flavour of frozen yogurt stored ~~for~~ at -20°C for 0-90 days under different treatments

The flavour scoresof frozen yogurt under different treatments are presented in Table 4 23a

The flavour scoresin frozen yogurt after freezing and hardening (0 day) under A-I A II A-III and A-IV were 42 17 40 83 41 63 and 42 43 respectively The corresponding scores treatment under B-I B-II B-III and B-IV were 42 07 41 30 40 40 and 42 43 respectively The scores under the treatments of I and IV (both in part A and B) were found to slightly higher than the II and III These differences between the treatments were found to be statistically not significant (Table 4 23b) Comparison of the flavour scores of frozen yogurt between 0 day and 90 days of storage indicate that in general no substantial change were noticed under majority of

Table 4 23a Score for flavour of frozen yogurt stored at -20°C for 0-90 days under different treatments

Treatments	Period (days) 0	15	30	45	60	75	90
A-I	42 17 ± 0 45	41 27 ± 0 61	41 17 ± 0 46	35 77 ± 6 43	42 53 ± 0 44	42 40 ± 0 23	40 13 ± 0 42
A-II	40 83 ± 0 53	41 73 ± 0 91	40 77 ± 0 42	42 53 ± 0 46	42 20 ± 0 29	42 70 ± 0 48	41 40 ± 0 31
A III	41 63 ± 0 84	41 37 ± 0 78	42 02 ± 0 72	42 15 ± 0 32	41 80 ± 0 42	42 51 ± 0 67	41 93 ± 0 35
A IV	42 43 ± 0 61	41 73 ± 0 56	41 40 ± 0 33	42 50 ± 0 28	41 28 ± 1 24	41 13 ± 0 73	40 83 ± 0 35
B-I	42 07 ± 0 37	40 87 ± 0 56	39 38 ± 0 17	41 43 ± 1 18	42 17 ± 0 84	39 85 ± 2 05	41 13 ± 0 62
B-II	41 30 ± 0 44	41 03 ± 1 19	39 07 ± 0 27	42 53 ± 0 31	41 40 ± 1 30	43 06 ± 0 45	40 57 ± 0 45
B-III	40 40 ± 0 63	40 90 ± 0 72	38 62 ± 0 20	42 33 ± 0 35	41 90 ± 0 33	41 69 ± 0 34	41 63 ± 0 38
B-IV	42 43 ± 0 62	40 40 ± 0 71	38 82 ± 0 44	42 17 ± 0 56	41 13 ± 1 24	41 58 ± 0 82	42 10 ± 0 20
CD			1 47				1 40

Each value is the mean of six replications

Table 4 23b Analysis of variance

Period (days)	Source	DF	MS	F
0	Between treatment	7	3 445	1 691 NS
	Within treatment	40	2 037	
15	Between treatment	7	1 250	0 337 NS
	Within treatment	40	3 709	
30	Between treatment	7	10 587	9 714 **
	Within treatment	40	1 090	
45	Between treatment	7	32 169	0 984 NS
	Within treatment	40	32 694	
60	Between treatment	7	1 469	0 322 NS
	Within treatment	40	4 567	
75	Between treatment	7	6 466	0 976 NS
	Within treatment	40	6 622	
90	Between treatment	7	2 787	2 826 *
	Within treatment	40	0 986	

NS - Non-significant * - Significant (P<0 05) ** - Highly significant (P<0 01)

Table 4.23c. t values

Treatments	0 Vs 15	0 Vs 30	0 Vs 45	0 Vs 60	0 Vs 75	0 Vs 90
A-1	1.25 NS	1.35 NS	0.99 NS	0.51 NS	0.64 NS	2.43 NS
A-II	0.79 NS	0.12 NS	3.53 *	3.05 *	2.54 NS	0.70 NS
A-III	0.32 NS	0.26 NS	0.59 NS	0.20 NS	1.43 NS	0.37 NS
A-IV	1.08 NS	1.79 NS	0.13 NS	0.91 NS	1.15 NS	1.82 NS
B-I	1.87 NS	1.15 **	0.68 NS	0.14 NS	0.93 NS	2.32 NS
B-II	0.23 NS	4.86 **	3.51 *	0.07 NS	2.15 NS	4.57 **
B-III	0.78 NS	2.40 NS	3.51 *	1.76 NS	1.65 NS	1.48 NS
B-IV	2.12 NS	3.45 *	0.27 NS	1.09 NS	1.01 NS	0.567 NS

NS - Non-significant * - Significant (P<0.05) ** - Highly significant (P<0.01)

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treatment The differences in the flavour score between 0 and 90 days were found to be statistically not significant under different treatment except under B-II A slight but significant reduction was noticed on 90 days of storage under B-II (Table 4 23c)

4 24 Score for the body and texture of frozen yogurt stored at -20°C for 0-90 days under different treatments

The body and texture score of frozen yogurt after freezing and hardening (0 day) under A-I A-II A-III and A-IV were 27 77 26 60 26 93 and 26 73 respectively The corresponding values under B-I B-II B III and B-IV were 27 93 27 06 27 53 and 26 83 respectively (Table 4 24a) The body and texture score in frozen yogurt (0 day) under A-II A-III and A-IV were lower than that of control (A-I) Similar trend was noticed under the treatments of part B However these differences between the treatments were statistically not significant (Table 4 24b)

The body and texture characteristic as judged by organoleptic evaluation remain steady upto 60 days of storage in almost all the treatments Thereafter a substantial reduction was noticed under all the treatments of part A and part B except A I Under A-I very less reduction in score was found on 75th day but higher reduction on 90th day

Table 4 24a Score for the body and texture of frozen yogurt stored at -20°C for 0-90 days under different treatments

Treatments	Period (days) 0	15	30	45	60	75	90
A-I	27 77 ± 0 53	28 96 ± 0 30	28 63 ± 0 22	27 10 ± 0 86	26 68 ± 0 43	27 08 ± 0 60	25 60 ± 0 24
A-II	26 60 ± 0 64	27 40 ± 0 29	27 50 ± 0 27	25 20 ± 0 77	26 60 ± 0 34	26 16 ± 0 34	25 90 ± 0 49
A III	26 93 ± 0 42	28 36 ± 0 33	28 30 ± 0 19	27 03 ± 0 55	25 73 ± 1 34	26 33 ± 0 73	25 26 ± 0 48
A-IV	26 73 ± 0 57	27 57 ± 0 58	27 36 ± 0 35	26 60 ± 0 30	26 73 ± 0 73	25 66 ± 0 49	25 66 ± 0 49
B I	27 93 ± 0 35	27 46 ± 0 33	27 10 ± 0 40	26 56 ± 1 18	27 91 ± 0 53	26 93 ± 0 49	25 93 ± 0 56
B-II	27 06 ± 0 31	25 80 ± 0 55	26 03 ± 0 42	25 20 ± 0 96	26 53 ± 0 54	26 63 ± 0 46	25 70 ± 0 13
B-III	27 53 ± 0 39	27 23 ± 0 73	27 56 ± 0 55	26 43 ± 0 54	27 06 ± 0 45	25 98 ± 0 43	25 70 ± 0 55
B-IV	26 83 ± 0 66	26 86 ± 0 31	26 76 ± 0 22	26 93 ± 0 61	25 16 ± 1 12	26 36 ± 0 75	25 83 ± 1 05
CD		1 59	1 21				

Each value is the mean of six replications

Table 4.24b. Analysis of variance

Period (days)	Source	DF	MS	F	
0	Between treatment	7	1.514	1.000	NS
	Within treatment	40	1.514		
15	Between treatment	7	5.397	4.275	**
	Within treatment	40	1.262		
30	Between treatment	7	4.053	5.487	**
	Within treatment	40	0.739		
45	Between treatment	7	3.533	0.987	NS
	Within treatment	40	3.579		
60	Between treatment	7	4.088	1.154	NS
	Within treatment	40	3.544		
75	Between treatment	7	1.352	0.728	NS
	Within treatment	40	1.856		
9fj	Between treatment	7	0.26/	0.139	NS
	Within treatment	40	1.917		

NS - Non-significant ** - Highly significant (P<0.01)

Table 4.24c. t values

Treatments	0 Vs 15	0 Vs 30	0 Vs 45	0 Vs 60	0 Vs 75	0 Vs 90
A-1	2.27 NS	1.60 NS	0.58 NS	1.59 NS	1.04 NS	3.79 **
A-II	1.47 NS	1.90 NS	1.49 NS	0.00 NS	0.72 NS	0.68 NS
A-III	1.93 NS	3.27 *	0.14 NS	0.88 NS	0.81 NS	1.99 NS
A-IV	1.10 NS	0.77 NS	0.29 NS	0.00 NS	1.17 NS	1.57 NS
B-I	1.53 NS	1.50 NS	1.36 NS	0.03 NS	1.41 NS	3.32 *
B-II	1.55 NS	1.61 NS	1.73 NS	1.02 NS	1.03 NS	4.96 **
B-III	0.36 NS	0.06 NS	1.49 NS	0.84 NS	2.82 *	3.56 *
B-IV	0.06 NS	0.14 NS	0.08 NS	2.44 NS	0.56 NS	0.59 NS

NS - Non-significant * - Significant (P<0.05) ** - Highly significant (P<0.01)

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Paired t test between the 0 and 90 days indicate that the reduction were statistically significant under B-I B-III and highly significant under A I and B-II (Table 4 24c)

4 25 Score for the melting quality of frozen yogurt stored at -20°C for 0-90 days under different treatments

Score for melting quality of frozen yogurt under different treatments stored for 0-90 days are presented in Table 4 25a

Melting quality score of frozen yogurt (0 day) under A-I A-II A-II and A IV were 4 70 4 66, 4 56 and 4 63 respectively Corresponding score under B-I B-II B III and B IV were 4 62 4 56 4 53 and 4 46 respectively

The score under A-II A-III and A IV eventhough statistically not significant (4 25b) but were slightly lower than A-I Similar trend was also found under the treatment of part B

The melting quality score remained steady through out the storage period and slight reduction was recorded on 90 days under A-I A II A III A IV B-I and B-II

The differences of melting quality score in frozen yogurt between 0 and 90 days samples under all the treatments were found to be not significant (Table 4 25c)

Table 4 25a Score for melting quality of frozen yogurt stored at 20°C for 0 90 days under different treatments

Treatments	Period (days)						
	0	15	30	45	60	75	90
A-I	4 70 ± 0 06	4 67 ± 0 06	4 77 ± 0 06	4 81 ± 0 07	4 43 ± 0 14	4 81 ± 0 05	4 46 ± 0 11
A-II	4 66 ± 0 09	4 73 ± 0 04	4 63 ± 0 03	4 80 ± 0 08	4 83 ± 0 16	4 63 ± 0 11	4 50 ± 0 08
A-III	4 56 ± 0 09	4 66 ± 0 06	4 63 ± 0 06	4 93 ± 0 04	4 73 ± 0 11	4 56 ± 0 13	4 33 ± 0 15
A-IV	4 63 ± 0 06	4 73 ± 0 04	4 70 ± 0 04	4 60 ± 0 16	4 75 ± 0 07	4 33 ± 0 18	4 33 ± 0 16
B I	4 62 ± 0 12	4 70 ± 0 04	4 73 ± 0 04	4 86 ± 0 09	4 58 ± 0 12	4 70 ± 0 06	4 17 ± 0 09
B II	4 56 ± 0 13	4 73 ± 0 04	4 80 ± 0 05	4 80 ± 0 09	4 50 ± 0 14	4 43 ± 0 11	4 46 ± 0 14
B-III	4 53 ± 0 06	4 50 ± 0 13	4 73 ± 0 04	4 80 ± 0 16	4 78 ± 0 07	4 60 ± 0 10	4 53 ± 0 16
B-IV	4 46 ± 0 12	4 70 ± 0 04	4 66 ± 0 06	4 86 ± 0 04	4 80 ± 0 08	4 58 ± 0 14	4 53 ± 0 11

Each value is the mean of six replications

Table 4.25b. Analysis of variance

Period (days)	Source	DF	MS	
	Between treatment	7	0.034	0.551 NS
	Within treatment	40	0.062	
15	Between treatment	7	0.036	1.328 NS
	Within treatment	40	0.027	
30	Between treatment	7	0.022	1.399 NS
	Within treatment	40	0.016	
45	Between treatment	7	0.057	0.855 NS
	Within treatment	40	0.066	
60	Between treatment	7	0.134	1.541 NS
	Within treatment	40	0.087	
75	Between treatment	7	0.134	1.525 NS
	Within treatment	40	0.088	
90	Between treatment	7	0.039	0.363 NS
	Within treatment	40	0.107	

NS - Non-significant

Table 4 25c t values

Treatments	0 Vs 15	0 Vs 30	0 Vs 45	0 Vs 60	0 Vs 75	0 Vs 90
A-I	0 61 NS	0 59 NS	1 78 NS	1 53 NS	1 40 NS	1 55 NS
A-II	0 50 NS	0 34 NS	1 02 NS	1 24 NS	0 27 NS	1 74 NS
A III	0 80 NS	0 79 NS	3 05 *	0 98 NS	0 00 NS	1 05 NS
A IV	2 23 NS	1 58 NS	0 15 NS	1 11 NS	1 86 NS	2 42 NS
B-I	0 68 NS	0 72 NS	1 98 NS	0 18 NS	0 75 NS	1 06 NS
B-II	1 18 NS	1 65 NS	1 16 NS	0 31 NS	0 72 NS	0 47 NS
B-III	0 19 NS	2 23 NS	1 39 NS	3 47 *	0 54 NS	0 00 NS
B IV	2 44 NS	1 93 NS	3 46 *	2 19 NS	0 52 NS	0 29 NS

NS - Non-significant * - Significant (P<0 05)

4 26 Score for colour and package of frozen yogurt stored at -20°C for 0-90 days under different treatments

The colour and package score in frozen yogurt stored for 0-90 days under different treatment are given in Table 4 26a

The colour and package score in frozen yogurt (0 day) under A-I A II A-III and A IV were 4 76 4 66, 4 66 and 4 /3 respectively The corresponding values under B-I B-II B-III and B IV were 4 76, 4 63 4 53 and 4 ›3 respectively These differences between the treatments were found to be not significant (Table 4 26b) The colou and package score of frozen yogurt remain steady upto 75 days of storage A highly significant decrease was observed on 90 days under A-I and significant decrease under B I Substantial decrease in colour and package score was noticed on 75 and 90 days under A IV The colour and package score uncer B-II was lower on 90 days in comparison to 0 day but it was found to be not significant (Table 4 26c)

4 27 Total score of frozen yogurt stored at 20°C for 0-90 days under different treatment

The total score of frozen yogurt stored at 20°C for 0-90 days are tabulated in Table 4 27a

Table 4 26a Score of colour and package of frozen yogurt stored at 20°C for 0 90 days under different treatments

Treatments	Period (days)						
	0	15	30	45	60	75	90
A I	4 76 ± 0 06	4 76 ± 0 08	4 76 ± 0 06	4 90 ± 0 03	4 58 ± 0 15	4 83 ± 0 03	4 36 ± 0 08
A II	4 66 ± 0 11	4 76 ± 0 03	4 70 ± 0 04	4 85 ± 0 09	4 43 ± 0 11	4 63 ± 0 13	4 50 ± 0 06
A III	4 66 ± 0 09	4 73 ± 0 06	4 80 ± 0 05	4 85 ± 0 09	4 63 ± 0 14	4 53 ± 0 12	4 56 ± 0 15
A-IV	4 73 ± 0 09	4 70 ± 0 11	4 63 ± 0 06	4 88 ± 0 04	4 71 ± 0 15	4 25 ± 0 13	4 30 ± 0 15
B I	4 76 ± 0 06	4 86 ± 0 06	4 83 ± 0 03	4 88 ± 0 09	4 61 ± 0 13	4 70 ± 0 06	4 31 ± 0 09
B II	4 63 ± 0 11	4 80 ± 0 05	4 70 ± 0 04	4 91 ± 0 06	4 60 ± 0 13	4 50 ± 0 11	4 26 ± 0 08
B-III	4 53 ± 0 09	4 66 ± 0 06	4 70 ± 0 06	4 75 ± 0 11	4 68 ± 0 15	4 58 ± 0 13	4 70 ± 0 09
B IV	4 63 ± 0 09	4 73 ± 0 04	4 86 ± 0 06	4 90 ± 0 04	4 71 ± 0 16	4 57 ± 0 13	4 43 ± 0 16

Each value is the mean of six replications

Table 4.26b. Analysis of variance

Period (days)	Source	DF	MS	
	Between treatment	7	0.038	0.664 NS
	Within treatment	40	0.057	
15	Between treatment	7	0.023	0.798 NS
	Within treatment	40	0.029	
30	Between treatment	7	0.038	2.078 NS
	Within treatment	40	0.018	
45	Between treatment	7	0.017	0.430 NS
	Within treatment	40	0.039	
60	Between treatment	7	0.051	0.402 NS
	Within treatment	40	0.127	
75	Between treatment	7	0.170	2.071 NS
	Within treatment	40	0.082	
TIT	Between treatment	7	0.134	1.57 2 NS
	Within treatment	40	0.086	

NS - Non-significant

Table 4.26. t values

Treatments	0 Vs 15	0 Vs 30	0 Vs 45	0 Vs 60	0 Vs 75	0 Vs 90
A-1	0.00 NS	0.00 NS	1.51 NS	0.94 NS	1.00 NS	5.47 **
A-II	0.88 NS	0.25 NS	1.14 NS	1.41 NS	0.15 NS	1.74 NS
A-III	0.46 NS	1.58 NS	1.33 NS	0.17 NS	1.08 NS	1.26 NS
A-IV	0.21 NS	0.80 NS	1.46 NS	0.07 NS	2.74 *	1.40 NS
B-I	1.16 NS	0.79 NS	1.55 NS	0.87 NS	0.67 NS	3.57 *
B-II	1.53 NS	0.79 NS	1.87 NS	0.18 NS	0.65 NS	2.10 NS
B-III	0.83 NS	1.27 NS	1.39 NS	0.68 NS	0.45 NS	0.95 NS
B-IV	1.00 NS	2.44 NS	2.16 NS	0.35 NS	0.27 NS	0.80 NS

NS - Non-significant * - Significant (P<0.05) ** - Highly significant (P<0.01)

Table 4 27a Total score of frozen yogurt stored at -20°C for 0-90 days under different treatments

Treatments	Period (days)						
	0	15	30	45	60	75	90
A-I	94 05 ± 0 78	94 60 ± 0 81	94 66 ± 0 51	94 93 ± 0 82	93 30 ± 1 05	94 08 ± 0 60	89 60 ± 0 70
A II	92 00 ± 0 95	93 70 ± 1 11	92 56 ± 0 45	92 63 ± 1 17	93 25 ± 0 60	93 15 ± 0 84	91 30 ± 0 83
A-III	91 63 ± 1 44	94 13 ± 0 77	94 70 ± 0 79	92 78 ± 0 98	90 58 ± 1 55	92 56 ± 1 40	91 00 ± 0 76
A IV	93 26 ± 0 73	93 83 ± 0 59	93 20 ± 0 49	93 46 ± 0 55	86 96 ± 5 12	90 56 ± 1 31	90 86 ± 1 31
B-I	94 23 ± 0 51	92 60 ± 0 75	90 80 ± 0 65	92 65 ± 2 31	94 21 ± 0 91	91 18 ± 2 00	90 86 ± 1 34
B-II	92 10 ± 0 50	91 46 ± 1 00	89 60 ± 0 62	91 85 ± 1 42	91 33 ± 2 60	93 38 ± 0 86	90 06 ± 0 46
B-III	91 06 ± 1 44	92 30 ± 0 84	90 65 ± 0 51	93 81 ± 0 84	93 50 ± 0 78	91 76 ± 0 80	91 56 ± 1 08
B IV	93 23 ± 1 03	91 70 ± 0 97	89 93 ± 0 58	94 33 ± 0 87	91 38 ± 2 45	91 23 ± 1 04	91 86 ± 1 20

Each value is the mean of six replications

Table 4 27b Analysis of variance

Period (days)	Source	DF	MS	F
0	Between treatment	7	8 087	1 382 NS
	Within treatment	40	5 854	
15	Between treatment	7	8 320	1 816 NS
	Within treatment	40	4 582	
30	Between treatment	7	25 246	12 144 **
	Within treatment	40	2 079	
45	Between treatment	7	6 228	0 681 NS
	Within treatment	40	9 146	
60	Between treatment	7	32 840	0 986 NS
	Within treatment	40	33 294	
75	Between treatment	7	9 252	0 786 NS
	Within treatment	40	11 767	
90	Between treatment	7	3 373	0 550 NS
	Within treatment	40	6 136	

NS - Non-significant ** - Highly significant (P<0 01)

Table 4.27b. Analysis of variance

Period (days)	Source	DF	MS	
0	Between treatment	7	8.087	1.382 NS
	Within treatment	40	5.854	
15	Between treatment	7	8.320	1.816 NS
	Within treatment	40	4.582	
30	Between treatment	7	25.246	12.144 **
	Within treatment	40	2.079	
45	Between treatment	7	6.228	0.681 NS
	Within treatment	40	9.146	
60	Between treatment	7	32.840	0.986 NS
	Within treatment	40	33.294	
75	Between treatment	7	9.252	0.786 NS
	Within treatment	40	11.767	
	Between Lredtment	7	3.373	0.550 NS
	Within treatment	40	6.136	

NS - Non-significant ** - Highly significant (P<0.01)

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A I (control)	Yogurt mix fortified with NDM and fermentation by thermophilus and bulgaricus
A II	Yogurt mix fortified with condensed cheese whey as a replacement of 50 per cent NDM and fermentation as in A I
A III	Yogurt mix fortified with condensed cheese whey as a replacement of 100 per cent NDM and fermentation as in A I
A IV	Yogurt mix fortified with whey protein dispersion for replacement of 100 per cent NDM and fermentation as in A-I
B I	- Yogurt mix fortified with NDM and fermentation by thermophilus bulgaricus and bifidobacteria
B II	Yogurt mix fortified with condensed cheese whey as a replacement for 50 per cent NDM and fermentation as B-I
B-III	Yogurt mix fortified with condensed cheese whey as a replacement for 100 per cent NDM and fermentation as B-I
B IV	Yogurt mix fortified with whey protein dispersion as a replacement for 100 per cent NDM and fermentation as B I

The total score for frozen yogurt (0 day) under treatment A I A-II A-III and A-IV were 94 05 92 00 91 63 and 93 26 respectively The corresponding values under B I B-II B-III and B IV were 94 23 92 10 91 06 and 93 23 respectively

The total score in frozen yogurt (0 day) under treatment A-IV was found to very close to the control (A-I) The score under A II and A III were slightly lower than control (A-I) Similar trend was maintained under treatments of part B The differences between treatment however were found to be statistically not significant (Table 4 27b)

During the storage the total score of frozen yogurt remained steady The comparison of total score in frozen yogurt between 0 and 90 day indicate no significant change under all the treatment except A-I and B-II (4 27c)

DISCUSSION

DISCUSSION

Yogurt is one of the most popular fermented milk product world wide It is distinguished from other cultured milk products by its typical flavour and aroma and its characteristic body and texture The yogurt starter culture viz S salivarius subsp thermophilus and L delbrueckii subsp bulgaricus in balanced ratio (1 1) is responsible for the typical green apple flavour of the product The body characteristics of the yogurt is dependent on the concentration of milk solids in the product

Non fat dry milk (NDM) is generally used for standardization of total solids in yogurt mix Due to increasing demand of casein and caseinates in food and baking industries (Southward 1989) the price of NDM has increased many fold in the last ~~the~~ 20 years

Cheese whey in condensed form can be an alternative for NDM in yogurt

In the present investigation, an attempt has been made to study the effect of incorporation of condensed cottage cheese whey and whey protein dispersion as a replacement for NDM on growth characteristic of normal yogurt starter culture

and bifidobacteria Effect of addition of condensed cheese whey and whey protein dispersion on biochemical and organoleptic characteristics of yogurt and frozen yogurt were also investigated

Survival of bifidobacteria in set and frozen yogurt and the influence of this organism on normal yogurt starter culture were studied Biochemical tests and sensory evaluation were undertaken to as ess the effect of incorporation of bifidobacteria in the product The data obtained are discussed under the following sub heads

Microbiological analyses

Biochemical analyses

Sensory evaluation

Microbiological analyses

5 1 Viable count of starter bacteria in set yogurt under different treatments

Milk is the most suitable growth medium for all types of micro organisms including the yogurt starter culture A variety of substrates like lactose protein fat and mineral matters are available in milk for their growth and multiplication A substantial amount of total nitrogen in milk occurs in a readily available form for bacterial

metabolism These include amino acid N, urea N and ammonia N These compounds are sufficient to initiate the growth of thermophilus which is one of the constituent of yogurt starter culture (Frank and Marth 1988) The proteolytic enzymes produced by bulgaricus are primarily responsible for casein breakdown and release of small peptides which are required for stimulation of thermophilus growth Thus the proteolytic activity of the bulgaricus is important for symbiotic growth of yogurt starter culture (Moon and Reinbold 1976)

A sufficient numbers of viable starter bacteria in cultured dairy products including yogurt are essential for their nutritional and therapeutic effects to consumers (Fernandes and Shahani 1989c)

Tamime and Robinson (1985) suggested that the yogurt should contain more than $100x10^6$ per ml of viable cells of thermophilus and bulgaricus at the time of consumption to have it s beneficial therapeutic effects

In the present investigation the optimum growth of thermophilus and bulgaricus were obtained under all the treatments (Table 4 1a) The thermophilus count varied from $3\ 21x10^9$ to $3\ 93x10^9$ cfu per ml under the treatments of part A in main trial

The replacement of NDM in yogurt by condensed whey solids and whey protein dispersion did not show any adverse effect on thermophilus count In fact a slight apparent increase in thermophilus count was noted in yogurt fortified with condensed cheese whey (under treatment A-II and A-III in main trial)

The thermophilus count in yogurt under treatment A-I and A-III in supporting trial (Table 4 2a) are also indicative of similar trend and confirm the findings of main trial Thus it could be concluded that the incorporation of condensed cheese whey had a stimulatory effect on thermophilus in yogurt

The present findings are in agreement with observation of Broome et al (1982) In their experiment it was found that the incorporation of whey protein concentrate in yogurt to replace part of NDM resulted in higher thermophilus TS2 count Hill et al (1974) reported that whey solids contain small peptides and glycomacropeptides These compounds are breakdown components of K-casein due to the action of rennet in the process of cheese making Moon and Reinbold (1976) found that some of the free amino acids and peptides released by bulgaricus by their proteolytic action were the stimulatory agents for the thermophilus

Apparent increase in thermophilus count in yogurt fortified with condensed whey in present investigation might be due to the availability of pre-formed peptides and amino-acids in excess quantity

Stimulatory effect of incorporation of condensed whey in yogurt was not noticed on the bulgaricus count The bulgaricus count in yogurt under different treatments was in the range of 2.36×10^9 - 3.91×10^9 cfu per ml (Table 4 1a) The bulgaricus count in yogurt prepared from the fortification of NDM (A-I) was found to be apparently higher than that of product fortified with condensed whey (A II and A-III) Moon and Reinbold (1976) reported that L bulgaricus possessed relatively high proteolytic activity and derive their nutrient requirement by breaking the α and β caseins It can therefore be assumed that the higher bulgaricus count in yogurt fortified with NDM in main trial might be due to availability of higher amount of casein than in the yogurt fortified with condensed whey The values of bulgaricus count under A-I and A III in supporting trial also indicate no stimulatory effect of condensed whey on the bulgaricus count The findings of Broome et al (1982) support the observation made under the present investigation

Eventhough it was found in the present experiment that the condensed whey had a stimulatory effect on the

thermophilus the counts of both thermophilus and bulgaricus in yogurt fortified with whey protein dispersion (under A-IV) were lower than under A-III The probable reason might be as under

Whey protein dispersion was prepared by the precipitation of proteins in whey by the combined action of heat and acid One per cent citric acid was used to lower the pH of the condensed whey to 4 6 The dispersion of whey proteins was collected by centrifugation and used for the fortification of yogurt mix The addition of acidified whey protein dispersion might have reduced the pH of yogurt mix (under treatment A-IV) before the start of incubation This might have resulted in the reduction of the incubation period required for the coagulation of the mix and attainment of desired level of pH

Thus low count particularly of bulgaricus in yogurt under A IV might be attributed to lesser period of incubation for attaining the desired pH If the incubation might have continued for some more time the thermophilus and bulgaricus would have been higher without further decrease in pH due to buffering action of whey proteins

The thermophilus count in yogurt under treatments of part B were in the range of $3\ 06 \times 10^9$ $4\ 26 \times 10^9$ cfu per ml

(Table 4 1a) The comparison of the values between the treatments of part A and part B indicate that the addition of bifidobacteria had not affected the count of thermophilus adversely Infact the count of thermophilus was found to be higher under treatment B I in comparison with A-I This finding is in agreement with that of Murti et al (1992) The thermophilus count in yogurt under treatment B-I in supporting trial was also higher than of A-I Thus the result of supporting trial confirmed the data obtained in main trial

It can be concluded that the thermophilus count was not affected by the presence of bifidobacteria in yogurt For the optimum growth of thermophilus the amino acids glycine histidine and glutamic acids were reported to be most essential (Bautista et al 1966) Collins and Hall (1984) stressed the importance of cysteine and ascorbic acid for the growth of bifidobacteria Poch and Bezkorovainy (1988) found that bovine casein digest and yeast extract were the most potential growth promoter for the bifidobacteria

From the findings of the present experiments it could be assumed that nutritional requirement of thermophilus and bifidobacteria are fulfilled by the proteolysis of casein by the bulgaricus and yogurt mix under all the treatments have sufficient nutrient to sustain optimum growth of thermophilus and bifidobacteria Perusal of the values in Table 4 1a also

indicate an apparent reduction in th thermophilus count in yogurt under treatments B II and B-III in comparison to B-I But is within the optimum range The composition of the raw material used for the fortification of yogurt mix is indicated in Table 3 1 The protein content in NDM was higher than that of condensed whey As such the yogurt mix under the treatments of B-I (fortified with NDM) might be having higher protein in comparison to B II and B-III (fortified with condensed whey) The apparently lower thermophilus count under treatment B II and B III might be due to non-availability of sufficient proteins and amino acids

The bulgaricus count under treatments of part B was in the range of $2\ 40x10^9$ - $3\ 46x10^9$ cfu per ml in main trial (Table 4 1a) and $1\ 66x10^9$ - $2\ 95x10^9$ per ml in supporting trial (Table 4 2a) In both the experiments the bulgaricus count in yogurt in presence of bifidobacteria was found to be in optimum range (as suggested by Tamime and Robinson 1985) However in comparison with the treatments under part A the count was found to be lesser under corresponding treatments of part B The bulgaricus count under A I in main trial was $3\ 91x10^9$ cfu per ml and under B-I it was $2\ 40x10^9$ cfu per ml The similar trend was also observed in supporting trial There was highly significant ($P<0\ 01$) reduction in bulgaricus count in yogurt under treatment B I in comparison with A I in

supporting trial These findings are in agreement with that of Murti et al (1992) This reduction might be attributed to competition for the nutrient with the bifidobacteria

The bulgaricus derive their major nitrogen source by hydrolyzing the casein (Moon and Reinbold 1976 Tamime and Robinson 1985) Poch and Bezkorovainy (1988) found that the bovine casein digest supported the optimum growth of many species of bifidobacteria in synthetic medium Poor growth of the bifidobacteria in the absence of bovine casein digest have been reported even if the media contain sufficient free amino acids

Low count of bulgaricus in presence of bifidobacteria under the treatment of B-I therefore may be due to non-availability of some of growth factor which may be common for both

Perusal of the values in Table 4 la indicate that the reduction of bulgaricus count was lesser under B III than that found under B-I This observation was confirmed in supporting trial (Table 4 2a) The bulgaricus count in yogurt under B III in supporting trial was significantly higher than B I and at par with A I (control) In otherwords the reduction of the bulgaricus count in the presence of bifidobactria in the sample of yogurt fortified with NDM was more than that of

condensed whey The probable reason for this might be utilization of whey proteins and other amino acids by the bifidobacteria for their growth thereby a reduction in the competition for the utilization of breakdown products of casein

The B bifidum count in set yogurt in main trial was in the range of $2\ 25 \times 10^9$ - $3\ 48 \times 10^9$ cfu per ml (Table 4 1a) The count under supporting trial was 2 80 $2\ 95 \times 10^9$ cfu per ml (Table 4 2a)

Ishibashi and Shimamura (1993) suggested that the fermented milk products must possess a minimum of 1×10^7 viable cells of bifidobacteria for their beneficial therapeutic effect to the consumers In the present investigation the bifidobacteria population was found to be optimum in the presence of thermophilus and bulgaricus

Bifidobacteria were reported to be a slow grower in milk and require some growth promoters for their optimum growth activity (Collins and Hall 1984) Bifidobacteria were also reported as nutritionally fastidious micro-organism and only limited number of organism could grow in minimal culture condition (Poch and Bezkorovainy 1988) Growth and acid production were quite different among the different strains and species of bifidobacteria The bifidobacteria of infant

origin were found to be comparatively faster in milk media and produce 0 65 per cent titratable acidity (Desjardins et al 1990)

However the optimum growth of bifidobacteria under present investigation was possible due to the associative action of thermophilus and bulgaricus The S salivarius subsp thermophilus were reported to produce enough carbon dioxide to support the growth of bulgaricus (Driessen et al 1982) It was also reported that L delbrueckii subsp bulgaricus posses significant proteolytic activity and were capable of releasing different peptides and amino acids by hydrolysing casein (Frank and Marth 1988) The compound released by thermophilus and bulgaricus might have stimulated the bidifobacteria growth under all the treatments of present investigation

The bifidobacteria count in yogurt under treatments of B II B III and B IV in main trial was apparently higher than that of B I The fortification of whey solids and whey protein dispersion in place of NDM might be the probable stimulatory factors for the bifidobacteria However the same effect was not observed under the treatments of supporting trial The count under treatment B-I and B-III was found to be very close The increase in the bifidobacteria count in presence of whey solids during the present investigation can

be corroborated with the findings of Marshal et al (1982) and Ventling and Mistry (1993)

Marshall et al (1982) found the optimum growth of B bifidum and B longum in ultrafiltered milk fortified with cheese whey protein and threonine

Ventling and Mistry (1993) found that ultrafilteration of skim milk to increase the protein concentration had beneficial effect on the growth of bifidobacteria The acid production and bifidobacteria count was higher in ultrafiltered milk

5 2 **Ratio between starter bacteria in set yogurt**

The thermophilus-bulgaricus ratio in yogurt should be 1 1 to produce optimum aroma and flavour (Tamime and Deeth 1980) The final ratio of thermophilus and bulgaricus is also indicative of symbiotic growth relationship between the two

The thermophilus bulgaricus ratio under different treatments was in the range of 0 56 1 21 (Table 4 3a) The ratios under treatments A I A-II A-III and B III were found to be optimum

Fortification of condensed whey in yogurt had no adverse effect on thermophilus bulgaricus ratio in majority of

treatments The low ratio under A IV was due to low count of bulgaricus in comparison with thermophilus

The presence of bifidobacteria had a adverse effect on thermophilus-bulgaricus ratio The ratio under treatment B I B II and B IV in main trial were lower than one (Table 4 3a) Highly significant (P<0 01) reduction of thermophilus bulgaricus ratio was found under treatment B-I in supporting trial (Table 4 4a) Lower growth of bulgaricus in presence of bifidobacteria was responsible for imbalance in the ratio

The ratio under B III in both the experiment were found to be around one and optimum The presence of whey proteins in the mix was indirectly responsible for restoring the proper balance of thermophilus and bulgaricus It can be concluded that condensed whey had no adverse effect on balance growth of normal yogurt starter culture Bifidobacteria however may cause adverse effect on the ratio especially in the yogurt prepared by fortification of NDM The incorporation of condensed whey to replace 100 per cent NDM supports the growth of thermophilus and bulgaricus in a balance form even in the presence of bifidobacteria Lower ratios under A IV and B IV were due to acidifying effect of whey protein dispersion Extended incubation period of yogurt mix under A IV and B IV may restore the proper balance

The thermophilus bifidobacteria ratio under different treatments were between 0 53-1 10 in main trial (Table 4 3a) and 0 86-0 93 in supporting trial (Table 4 4a) Low ratio was found under B I This was because of low bifidobacteria count in yogurt fortified with NDM

The bulgaricus-bifidobacteria ratio were in the range of 0 94-1 37 under the treatments of main trial and 0 94 1 77 under the treatments of supporting trial (Table 4 3a and 4 4a respectively) The importance of thermophilus bulgarius ratio were pointed out in literature for symbiotic growth proteolysis and production of flavour compound (Tamime and Robinson 1985) No reports are available regarding optimum ratio between the population of thermophilus bifidobacteria and bulgaricus-bifidobacteria However Hoover (1993) and Ishibashi and Shimamura (1993) had stressed the importance of bifidobacteria in cultured milk in a range of 10^7 10^8 to have a beneficial therapeutic effect

In the present experiment optimum growth of bifidobacteria was obtained in the presence of normal yogurt culture However the incorporation of condensed whey to replace 100 per cent NDM was found to be the best preposition as it supported the balance growth of all the three organisms

5 3 Effect of freezing and hardening on viable starter count in yogurt under different treatments

The comparison of viable count of thermophilus bulgaricus and bifidobacteria between set and frozen yogurt (Fig 2 3 and 4) indicate the reduction during the process of freezing and hardening These observations were confirmed in supporting trial (Fig 5 6 and 7) A highly significant reduction in the count of all three starter bacteria during freezing and hardening were recorded in supporting trial (Table 4 6a) Reduction in the thermophilus bulgaricus and bifidobacteria count under all the treatments however was limited to one logarithmic unit The thermophilus bulgaricus and bifidobacterial count in frozen yogurt (0 day) were in the range of 0 94 x 10^9 - 2 45 x 10^9 1 30 x 10^9 2 63 x 10^9 and 1 54 x 10^9 2 63 x 10^9 cfu per ml respectively in main trial (Table 4 5a) These values are found to be in optimum range as recommended by Tamime and Robinson (1985) Ishibishi and Shimamura (1993) for using this organism as a dietary adjuncts

Similar range of reduction in starter culture count during the process of freezing and hardening was recorded by Modler et al (1990) Mashayekh and Brown (1992) and Heknat and McMohan (1992)

Speck and Ray (1977) reported that gram positive bacteria were generally less susceptible to freeze injury than gram negatives Marth (1973) and Sheu et al (1993) reported that casein sucrose, lactose could act as the cryoprotective substances and protect the starter bacteria during freezing and hardening Sheu et al (1993) also found that loss of viable bulgaricus cells could be decreased by the microentrapment in calcium alginate beads Addition of six per cent glycerol further enhanced the protective effect

The frozen yogurt mix under different treatment in the present investigation was having more than 13 per cent SNI 10 per cent fat 0 25 per cent glycerol monosterate (GMS) 0 25 per cent sodium alginate and 15 per cent sugar

It can be concluded that the damage to the starter bacterial cells during the freezing and hardening of yogurt was minimum This might be due to the presence of hydrocolloids like the sodium algirate and cryoprotective substances like casein lactose and ugar The extra amount of calcium might have formed a alginate beads to protect the bacterial cells against the freezing damage

5 4 Effect of storage of frozen yogurt at 20°C for 0-90 days on S salivarius subsp thermophilus count under different treatments

The perusal of Table 4 7a indicate that there were a linear decrease in the thermophilus count in frozen yogurt under all the treatments during the period of storage of 90 days The rate of decrease was very slow upto 30 days of storage but the count declined drastically between 30 and 45 day and remained steady till the end of 90 days (Fig 8a and 8b) However even after 90 days the population of thermophilus in the frozen yogurt was in substantial number At the end of 90 days of storage the thermophilus count und r different treatment were in the range of 0 25 x 10^9 0 35 x 10^9 cfu per ml Comparison of the count between 0 and 90 day indicated that the decrease was limited to only one logarithmic unit Similar trend was also recorded in the supporting trial (Table 4 10a)

Perusal of the t values in Table 4 10c indicate the highly significant ($P<0.01$) reduction in the thermophilus count during the storage of frozen yogurt for 30 days at -20°C under majority of the treatments in supporting trial

5 5 Effect of storage of frozen yogurt at -20°C for 0-90 days on L delbrueckii subsp bulgaricus count under different treatments

The data in Table 4 8a indicate a gradual reduction in bulgaricus count in frozen yogurt during storage The rate of decrease of bulgricus count under all the treatments was uniform throughout the storage period (Fig 9a and 9b) After 90 days of storage the bulgaricus count in frozen yogurt were in the range of 0 18 x 10^9 0 45 x 10^9 cfu per ml The decrease in bulgaricus from beginning of the storage period till 90 days was also limited to about one log cycle

Statistical analysis of differences in the bulgaricus count between 0 and 90 days were found to be significant under A-III A-IV B III and B-IV of main trial (Table 4 8c) Significant reduction in bulgaricus count during storage of frozen yogurt at -20°C for 30 days was also recorded under the treatments of A I A III B I and B-III in supporting trial (Table 4 10c)

5 6 Effect of storage of frozen yogurt at -20°C for 0-90 days on B bifidum count under different treatments

Gradual reduction in the count of B bifidum during the storage of frozen yogurt was also observed under all the treatments studied The rate of reduction of bifidobacteria

count was comparatively high between 0 and 15 day of storage (Fig 10) After 15 days decrease in the count was steady After 90 days of storage the count was in the range of 0 07 x 10^9 - 0 13 x 10^9 cfu per ml (Table 4 9a) The differences in the bifidobacteria count between 0 and 90 days were found to be statistically significant under B-II B-III and B-IV (Table 4 9a) The reduction in the count after 90 days of storage was however limited to less than two log cycle The comparison of bifidobacteria count at 0 and 30 days under treatment of supporting trial were found to be highly significant (Table 4 10c)

From the above results it could be concluded that there was a gradual decrease in the count of starter bacteria during the storage But still sufficient numbers were f und at the end of 90 days of storage to have its beneficial effect after consumption The decr ase in the count of thermophilus and bulgaricus were of only one log cycle and that of bifidobacteria was between one to two log cycle

The findings of Modler et al (1990) Holcomb et al (1991) Hekmat and McMohan (1992) Laroia and Martin (1 91) and Mashavekh and Brown (1992) support the observation made in the present investigation

Modler et al (1990) found a significant reduction in the population of B longum B brevi and B infantis in cultured ice cream by one log cycle during storage for 70 days at -30°C

Holcomb et al (1991) reported the viability and growth of B bifidum and L acidophilus in soft serve frozen yogurt at -5°C during the storage period of six hours

Laroia and Martin (1991) reported B bifidum population was 3 7 x 10^6 4 0 x 10^6 cfu/ml in frozen yogurt after eight weeks of storage at -29°C

Hekmat and McMohan (1992) found that after 17 weeks of storage of cultured ice cream at -29°C the B bifidum count were 1 x 10^7 cfu per ml

Mashayekh and Brown (1992) indicated that if cultured ice-cream were stored at -29°C for more than one month it would still have the viable cells of thermophilus and bulgaricus in the range of 10^6 10^7

Above reports support the present findings and also that bifidobacteria could be incorporated successfully in frozen yogurt and the product could be stored for 90 days without substantial reduction in starter bacterial population

5 7 Coliform, yeast and mould count in set yogurt under different treatments

Coliform count in the set yogurt under different treatments was in the range of 1 66-5 cfu per ml (Table 4 11)

The yeast and mould count in the set yogurt under different treatments was in the range of 3 33 - 11 66 cfu per ml

The count of these organi ms in fermented dairy product was carried out to assess the standard of hygienic measures adopted during their preparation Indian standards (1973) specified the limits for coliform yeast and mould count in fermented milk product The coliform count in the product should not be more than 10 and yeast and mould 100 per ml

The count of coliform yeast and mould in set yogurt under different treatment in the present investigation were found to be within the limit specified by IS (1973)

Biochemical analyses

5 8 Comparison of titratable acidity and pH between set and frozen yogurt under different treatments

The most important fermentative reaction used in dairy

processing is the homofermentative conversion of lactose to lactic acid The efficient manufacture of high quality fermented product including yogurt requires a rapid and consistent rate of lactic acid production (Frank and Marth 1988) The lactic acid gives the sharp acid taste to yogurt and contributes to the typical flavour (Tamime and Robinson 1985) The lactic acid also helps to destabilize the casein micelle and this lead to coagulation of milk protein and formation of the yogurt gel Production of lactic acid by starter culture is considered as one of the criteria to judge its activity

S salivarius subsp thermophilus and L delbrueckii subsp bulgaricus are homofermentative organism In the mixed culture they are capable of producing optimum range of lactic acid under favourable conditions of incubation (i e at 30°C for long set or 42°C for short set method)

The yogurt coagulum of desirable firmness could be obtained by incubating the mix to a pH of 4 6 - 4 7 (Tamime and Robinson 1985) Mild yogurts of pH 4 4 4 6 and acidity of 0 9 per cent are being favoured by the consumers Considerable efforts being made by commercial companies to maintain these pH values during the period between manufacture and consumption (Marshall 1993)

Yogurt having pH of 4 4 4 6 and acidity 0 9 - 1 0 per cent are known to maintain a proper balance of thermophilus and bulgaricus (Tamime ard Robinson 1985) The optimum level of acidity (around one per cent) is also found to be important to prevent the growth of pathogens (Schaack and Marth 1988)

In the present experiment the titratable acidity under the treatments of part A and part B was in the range of 0 93 1 02 per cent equivalent lactic acid at fixed pH of 4 6 (Table 4 12a) The minor variations in the acidity between the treatments can be attributed to different buffering capacity of the yogurt mix

The bifidobacteria were reported to produce lactic acid and acetic acid in 2 3 ratio (Scardovi 1986) The values of titratable acidity obtained under treatments of part B of the present investigation include both lactic and acetic acid

The titratable acidity in the frozen yogurt after freezing and hardening (0 day) under different treatment was in the range of 0 85 0 94 per cent equivalent lactic acid (Table 4 12a) and pH in the range of 4 69 4 84 (Table 4 14a)

The set yogurt was used as a base for the preparation of frozen yogurt after standardization of fat and total

solids Cream sugar sodium alginate and glycerol monostearate (GMS) were blended with set yogurt after incubation and cooling The titratable acidity and pH were measured after batch freezing and hardening at 20°C

The comparison of titratable acidity and pH between set and frozen yogurt indicate a slight reduction in acidity and a slight increase in pH of the latter

Under all the treatments the titratable acidity was slightly lower and pH slightly higher in frozen yogurt than the corresponding treatments in set yogurt The effect may be due to dilution of acidity by addition of cream and sugar

5 9 Titratable acidity and pH in frozen yogurt storage at -20°C for 0 90 days under different treatments

A slight increase in acidity and corresponding decrease in pH in frozen yogurt under different treatments were recorded during its storage for 90 days (Table 4 13a and 4 14a) The differences in the acidity in frozen yogurt between 0 and 90 day under all the treatments however was found to be not significant

In a survey conducted by Kosikowski (1981) to assess the chemical and microbiological characteristic of commercial frozen yogurt it was found that only 50 per cent of total

samples analysed were having a desired pH of 4 0 - 4 55 and 0 8 - 1 35 per cent titratable acidity The samples with pH 4 0 - 4 5 were having optimum lactic count The samples with high pH and low acidity were devoid of optimum lactic count and lack the typical yogurt flavour

5 10 Contents of total solids in set yogurt under different treatments

The percentage of total solids in set yogurt under different treatments were in the range of 20 86 21 73 (Table 4 15a) To maintain the proper body and texture of the yogurt Tamime and Robinson (1985) recommended that the total milk solids in the mix should be 15 16 per cent Desired levels of total solids can be achieved by fortification with NDM or by heat concentration or by membrane processing namely ultrafilteration and reverse osmosis

In the present investigation the standardization of mix for total solids was carried out by fortification with NDM condensed cottage cheese whey or with whey protein dispersion

Solid not fat levels of yogurt mix under all the treatments were maintained at 13 per cent Mix was standardized to three per cent fat and sugar was added at rate of five per cent

5 11 Contents of non-protein nitrogen (NPN) in set yogurt under different treatments

Proteolysis in the set yogurt under different treatment in the present investigation was measured in terms of content of NPN and tyrosine value

The content of NPN in set yogurt under A I A II A III and A-IV were 63 03 52 53 46 69 and 60 69 mg per 100 g respectively (Table 4 16a) The corresponding values under B I B-II B III and B IV were 51 36 58 36 49 61 and 63 03 mg per 100 g Wide variations in the values for NPN were observed between treatments however the differences were found to be statistically not significant The over all mean of the treatments under part A was 55 74 and that of treatments under part B was 55 59 This indicate that the NPN content of yogurt under part A and part B were almost same The combined mean of part A and part B under treatments I II III and IV were 57 20 55 45 48 15 and 61 86 respectively (Table 4 16a) The differences in the values might be due to the nature of fortification of yogurt mix The data obtained were regrouped according to the method of Mead (1988) to evaluate the effect of treatment on the content of NPN From the pooled analysis it was found that the NPN content in yogurt prepared with fortification of whey protein dispersion was significantly higher than that of yogurt with condensed

whey at replacement level 100 per cent Content of NPN in yogurt prepared by fortification of whey protein dispersion and with NDM were at par with each other

The analysis of raw material used for the fortification of mix (Table 3 1) indicate a higher levels of total proteins in NDM than condensed whey The yogurt mix with fortification of condensed whey (under treatments II and III) might be having low levels of total proteins in comparison to mix with NDM and whey protein dispersion (under treatments I and IV respectively) The low values of NPN under treatments II and III could be attributed to low level of total protein in the mix

The highest NPN content was recorded under treatment IV The presence of higher amount of whey proteins in this mix might be attributed for higher proteolysis

Alm (1982c) reported that NPN content of yogurt was 45 mg per 100 g and Shankar et al (1983) found that the NPN amounted to 7 8 per cent of total nitrogen in yogurt Tamime and Robinson (1985) reported that the free amino acids in yogurt varied from 18 77 33 06 mg per 100 g

It can be concluded that the presence of bifidobacteria in the yogurt did not have any influence on the

The tyrosine values in set yogurt under treatment A I A II A III and A IV were 0 18 0 20 0 17 and 0 24 mg per g respectively (Table 4 17a) The corresponding values under B I B II B III and B IV were 0 20 0 21 0 19 and 0 24 mg per g respectively The values under part A were very close to the corresponding treatment of part B and values of A IV and B IV were exactly same

The data of NPN content and tyrosine values in the fermented products indicate the nature of proteolysis Whey solids and whey protein dispersion contain certain small peptide derived from αS_1 casein and also glycomacropeptides from the action of rennin on K casein (Hill et al 1974) The thermophilus possessed good peptidase activity and whey proteins were considered as a ready source for their growth The higher proteolysis under the treatment IV (of both A-IV and B IV) can be attributed to the nature of protein

The proteolysis in yogurt was reported to be dependent upon the strains of starter culture and the ratio between thermophilus bulgaricus (Tamime and Deeth 1980)

Shankar et al (1983) reported the tyrosine value in yogurt as 23 1 mg per 100 g The amount of amino acids found in yogurt was reported to be a balance between their release due to proteolysis and their utilization (Slocum et al 1988) It was found that amount of free amino acids in the milk after the fermentation by a single culture of bulgaricus were higher than in the product in which fermentation was carried out by mixed culture of thermophilus and bulgaricus (Slocum et al 1988) Their finding indicate that the utilization of amino acids by thermophilus after their release by the proteolytic action of the enzymes produced by bulgaricus

The bifidobacteria were reported to have a very weak proteolytic activity however considerable strain variation were also reported (Kurmann 1988) Misra and Kuila (1991) reported the tyrosine values among th different strains of B bifidum The tyrosine value in milk fermented by B bifidum isolated from the infant fecal samples was in the range of 202 286 microgram per g after 24 hours of incubation The values with the strains from NDRI Karnal and National Collection of Dairy Organism (NCDO) Reading were 245 371 5 microgram per g

The tyrosine value in frozen yogurt (0 day) under treatment A-I A II A-III and A-IV were 0 17 0 17 0 22 and

0 25 mg per g respectively (Table 4 17a) The corresponding values under treatment B I B II B III and B-IV were 0 19 0 19 0 23 and 0 26 mg per g respectively

Comparison of tyrosine values between set and frozen yogurt indicate the differences in the values were statistically not significant under all treatments except A-II

The tyrosine value in frozen yogurt (0 day) under treatment A II was significantly lower than that of set yogurt The variations in the tyrosine value under different treatments between set and frozen yogurt might be due to the addition of cream and sugar for the standardization of frozen yogurt mix

5 13 Tyrosine values in frozen yogurt stored at 20°C for 0 90 days under different treatments

The tyrosine values in frozen yogurt under all the treatments gradually increased during the period of storage Sharp increase in tyrosine value was noticed between 0 and 15 days of storage under A I A II B-I and B II The range of tyrosine value under different trea ments on 15th day of storage were 0 21 0 25 mg per g After 15 days the rate of increase was slow The tyrosine value in frozen yogurt after 90 days varied from 0 27 0 28 mg per g The statistical

analysis for comparison of tyrosine values in frozen yogurt between 0 and 90 days of storage indicate a significant increase under A II B II and B III Eventhough increase in tyrosine value during storage was found to be statistically not significant under the remaining the treatment it is clear from the data tabulated in Table 4 18a that there were gradual increase in the values under all the treatment

It is an established fact that metabolic activity of starter culture ceases at the storage temperature of 20°C and hence the possibility of proteolysis by the micro organism can be completely ruled out The perusal of the data in the Table 4 7 - 4 9 however indicate that there were gradual reduction in the count of thermophilus bulgaricus and bifidobacteria during the storage of frozen yogurt This indicate that there was a continuous lysis of the bacterial cells due to the frozen storage The gradual increase in the tyrosine values in frozen yogurt could be correlated with the bacterial lysis during the storage The release of the proteins due to the break down of the bacterial cells might have increased the tyrosine value in the frozen yogurt

Keeney and Kroger (1987) pointed out that freezing as such would not have any significant effect on milk proteins but on frozen storage the stability of calcium caseinate phosphate complex was affected The effect of frozen storage

on protein stability was also found to be dependent on storage temperature and duration of the storage It was further reported that interaction among milk component were not completely inhibited during the frozen storage The increase in tyrosine value during storage may not be due to complete destabilisation of casein but some minor mechanical damage to the casein micelles cannot be completely ruled out Therefore it could be concluded that the increase in tyrosine value of frozen yogurt during storage is due to (i) to lysis of bacterial cells and (ii) mechanical damage to milk proteins

5 14 Contents of diacetyl and acetaldehyde in set yogurt under different treatments

The diacetyl is the flavour producing compound and in combination with acetaldehyde is responsible for characteristic green apple flavour of the yogurt The diacetyl content in set yogurt in the present experiment under different treatments are given in Table 4 19a

The content of diacetyl under A-I A II A III and A-IV were 13 92 12 80 10 00 and 19 00 ppm respectively (Table 4 19a) The concentration of the diacetyl under these treatments in the present experiment were found to be higher than earlier reported values Green and Manning (1982) reported that diacetyl concentration rarely exceed 0 5 ppm in

yogurt Shukla (1986) reported a value of 10 75 ppm in yogurt prepared from buffalo milk Prasad (1990) recorded the diacetyl concentration in yogurt ranged from 3 2 - 8 0 ppm

Strain differences in starter ulture might be one of the reason for variations in the content of diacetyl in present experiment and those of the reported values

The diacetyl concentration under A-IV and B IV were found to be higher than control The yogurt mix under the treatment of A IV and B-IV were fortified with whey protein dispersion One per cent citric acid was used for acidification of condensed whey to bring down the pH to 4 6 and for getting the whey protein dispersion

Cogan (1981) and Mellerick and Cogan (1981) reported that the presence of excess citrate in the growth media suppressed both diacetyl reductase and acetoin reductase activity thereby allowing the accumulation of diacetyl and acetone in the product In the light of the observations made by Cogan (1981) and Mellerick and Cogan (1981) it could be inferred that the apparently higher diacetyl content in the yogurt prepared from the mix fortified with whey protein dispersion (under treatments A-IV and B-IV) may be due the p esence of the residual citrate

The diacetyl concentration in yogurt under the treatments B I B II B III and B IV were 10 17 10 13 7 83 and 15 17 ppm respectively These values were found to be lower than the corresponding concentrations under A I A II A III and A IV Similar observations were also recorded by Murti et al (1992) In their experiment the diacetyl concentration in yogurt was 0 66 ppm and it was reduced to 0 25 ppm due to supplementation B bifidum as a third starter culture

Diacetyl is reported to be produced by the lactic acid bacteria during glucose and citric acid metabolism by two different path ways (Keenan and Lindsay 1968) Pyruvic acid is the intermediate product in fermentation of both glucose and citric acid The utilization of pyruvic acid by lactic acid bacteria for the production of different flavour compounds including the lactic acid is well documented (Keenan and Lindsey 1968 Seitz 1990) The main flavouring compound originating from pyruvic acid metabolism besides lactic acid are acetaldehyde ethanol diacetyl and acetoin

Acetaldehyde thiamine pyrophosphate (TPP) complex is produced by decarboxylation of pyruvate The reaction require a d valent metal either Mg^{+2} or Mn^{+2} and the enzyme pyruvate carboxylase The acetaldehyde TPP complex was found to combine with acetyl COA to produce diacetyl Diacetyl

synthetase act as a catalyst in this reaction (Seitz et al 1963) Cogan (1981) reported that the diacetyl can be reduced to acetoin by the enzyme diacetyl reductase

Frank and Marth (1988) reported that the concentration of diacetyl in a product was dependent on the availability of acetyl COA Low concentration of diacetyl in fermented products could also be due to its conversion into acetoin because of high diacetyl-reductase activity

Potential sources of diacetyl reductase in the cultured dairy product include starter culture organism as well as contaminants such as coliform yeast mould and other non-starter lactobacilli (Steitz et al 1963 Keenan and Lindsay 1968 Wang and Frank 1981)

Bifidobacteria are characterised by a unique form of carbohydrate metabolism (Frank and Marth 1988) De Varies and Stouthammer (1967) studied the pathway of glucose fermentation in 17 strains of B bifidum They have reported the absence of aldolase an enzyme unique to glycolysis for homofermentative organism ard glucose 6-phosphate dehydrogenase in all the strains of bifidobacteria The latter enzyme was the characteristic of all the homofermentative lactic acid bacteria In the cell free

extract of all the 17 strains studied activity of fructose 6 phosphate phsophoketalase was demonstrated by De Varies and Stouthammer (1967)

In a separate experiment De Varies and Stouthammer (1968) found that acetate L(+) lactate ethyl alcohol and formate were the fermentation end products of glucose galactose lactose mannitol and xylose metabolism by bifidobacteria The variation in concentration of these end products were also reported to be dependent on the strains and also with substrate used with the particular strain They found that the amount of lactate formed was different in glucose and lactose fermentation with the same strain

In their experiment they were unable to detect acetoin and pyruvate after the fermentation They explained the reasons for the absence of acetoin and pyruvate in the media after the carbohydrate fermentation by bifidobacteria as under The pyruvate formed from glu ose via the fructose 6 phosphate phosphoketolase route was partly reduced to lactate and partly split to acetyl phosphate and formate They also pointed out that bifidobacteria convert pyruvate formed as an intermediate product by the two pathways The first was reduction of pyruvate to L(+) lactate The second path was cleavage of pyruvate into acetyl phosphate and formate Part of acetyl phosphate formed was reduced to ethyl alcohol

In the light of foregoing observations it could be inferred that the low concentrations of diacetyl in the yogurt under the treatments of part B in the present investigtion might be due to the utilization of pyruvic acid by the bifidobacteria for the production acetate lactate formate and ethanol Some of the pyruvic acid might have been utilized for the production of diacetyl by the thermophilus and bulgaricus

The acetaldehyde concentration in yogurt under treatment A I A-II A-III and A-IV were 36 50 33 67 43 04 and 31 05 ppm respectively (Table 4 19a) Acetaldehyde is a very important flavour compound and is responsible for characteristic flavour of yogurt Kang et al (1988) suggested that the acetaldehyde at the level of 23 41 ppm gave an optimum flavour to yogurt Great variations in acetaldehyde concentration in yogurt were reported earlier Hamdan et al (1971) reported a value of 22 26 ppm and Abrahamsen et al (1978) 17 1 ppm acetaldehyde in yogurt Green and Manning (1982) found the acetaldehyde concentration of 23-41 ppm in yogurt

Tamime and Robinson (1985) opined that the concentration of acetaldehyde in fermented milk product was depended on the starter culture incubation temperature and levels of total solids in the mix The content of

acetaldehyde obtained for different treatments in the present experiment are within the range of reported value except under A III The acetaldehyde content in yogurt prepared from fortification of condensed whey as a replacement for 100 per cent NDM (A III) was significantly higher than that of other treatments

Acetaldehyde is reported to be formed during glucose metabolism by lactic acid bacteria (Lees and Jago 1978) The glucose was metabolized to acetaldehyde by the yogurt starter bacteria by the activity of enzyme aldehyde dehydrogenase Hickey et al (1983) reported that acetaldehyde was also produced from amino acid threonine Threonine aldolase which was reported to be present in L delbrueckii subsp bulgaricus was responsible for conversion of threonine to acetaldehyde The higher level of acetaldehyde under treatment A III in present investigation may be attributed to the availability of extra amount of free threonine in the mix by the fortification of condensed whey Even though the nature of available proteins and amino acids were similar in yogurt under treatments A III and A IV still lower concentration of acetaldehyde was recorded under latter The data from Table 4 1a indicate the lower cour of thermophilus and bulgaricus under treatment A IV than that of A III Therefore the probable reason for ower concentration of

acetaldehyde in yogurt prepared from fortification with whey protein dispersion than that with cordensed whey was due to lower count of thermophilus and bulgaricus under treatment A-IV

The content of acetaldehyde in yogurt under B-I and B III were found to be substantially lower than the that of A I and A III The concentration under A I and A III were 36 50 and 43 04 ppm respectively The corresponding values under B I and B III were 31 88 and 34 52 respectively

Similar findings regarding the reduction of acetaldehyde by the presence of bifidobacteria was recorded by Murti et al (1992) The concentration of acetaldehyde was reduced from 38 95 to 6 ppm and ethanol levels increased from 0 35 to 0 55 ppm in yogurt supplemented by addition of a culture of bifidobacteria

Lees and Jago (1978) reported the reduction of acetaldehyde into ethanol by the action of enzyme alcohol dehydrogenase by certain starter culture The characteristic flavour of yogurt was reported to be due to accumulation of high concentration of acetaldehyde in the product It was reported that the acetaldehyde was not reduced by normal yogurt starter culture as the enzyme alcohol dehydrogenase was not present in both the organisms

The low concentration of acetaldehyde in the product fermented by bifidobacteria alone or in combination of starter culture may also be attributed to the presence of alcohol dehydrogenase in B bifidum (De Varies and Stouthammer 1968)

The lower concentration of acetaldehyde in yogurt under B I and B III in present experiment could be due to reduction of part of the acetaldehyde to ethanol by the enzyme alcohol dehydrogenase produced by bifidobacteria

Another important reason for low levels of acetaldehyde under treatment B I could be the substantially low count of bulgaricus as compared to A I (Table 4 1a) But in yogurt under treatment B-III had sufficiently higher bulgaricus count but still the lower concentration of acetaldehyde was recorded Thus higher number of bifidobacteria in yogurt under B III in comparison with B I may be responsible for the reduction of the part of acetaldehyde to ethanol

Marshall et al (1982) reported that the fortification of threonine stimulated the bifidobacteria growth in the ultrafiltered milk They also found production of acetaldehyde by the B bifidum B infantis B longum and B adolescentis in the product w ere anged from 27-39 ppm

Thus the above findings reflect that the acetaldehyde concentration was dependent on the types of protein in the mix the population of bulgaricus and bifidobacteria The final levels of acetaldehyde is the balance between its production and its reduction into ethanol

5 15 Content of acetic acid in set yogurt and frozen yogurt stored at -20°C for 0, 45 and 90 days under different treatments

The acetic acid content in set yogurt under the treatments of part A were not detectable by the gas chromtographic method used in present experiment The values of the acetic acid in yogurt under the treatment B-I B II B III and B IV were 0 21 0 14 0 16 and 0 08 per cent respectively (Table 4 20) Production of acetic acid in traces were reported for most of the homofermentative lactobacilli (Kandler and Weiss 1986) during the anaerobic fermentation of glucose Different species of bifidobacteria produce acetic acid and lactic acid in the ratio of 3 2 by the fermentation of glucose (Scardove 1986) The fermentation involves two phosphoketolase enzymes one specific for fructose 6 P which produce acetyl pho phate and erythrose 4 phosphate and the other specific for xylulose 5 P which produce acetyl P and glyceraldeyde 3 P This path way yields five moles of ATP for every two moles of glucose metabolized

As such it is more efficient than homolactic fermentation (Frank and Marth 1988)

The higher levels of acetic acid production in yogurt under the treatments of part B in comparison with that of part A in present investigation is the indicative of the normal glucose metabolism by bifidobacteria in presence of thermophilus and bulgaricus

The product prepared from fortification of NDM in yogurt mix (under treatment B I) produce apparently higher acetic acid than product fortified with condensed whey (B II and B III) The lowest level of acetic acid was found in yogurt prepared by fortification of whey protein dispersion (under B IV) In the light of above it could be suggested that the nature of proteins of the mix may have some influence on the production of acetic acid It may be interesting to note that yogurt under B IV (fortified with whey protein dispersion) produced substiantially higher amount of diacetyl and very low acetic acid Both these compounds are formed during glucose metabolism but from two different path ways

De Varies and Stouthammer (1968) found that the concentration of acetic acid was deperdent on the strains of bifidobacteria and the substrate used They recorded

different values for acetic acid by the metabolism of glucose galactose and lactose

Strain difference for production of acetic acid and lactic acid by the bifidobacteria was also reported by Desjardins et al (1990) Different strains of B bifidum produced acetic acid in the range of 3 53 - 12 10 millimoles

Ventling and Mistry (1993) found that concentration of protein by ultrafilteration of skim milk increase the production of acetic acid by the bifidobacteria They recorded the acetic acid level in normal skim milk with 3 15 per cent protein as 5 61 millimoles However by increasing the concentration of protein to six per cent by ultrafiltration the concentration of acetic acid was increased to 9 37 millimoles

The variation in the concentration of acetic acid under the different treatments in the present experiment may therefore be attributed to nature and levels of protein in the mix

The content of acetic acid in frozen yogurt immediately after freezing and overnight hardening (0 day) under B I B II B-III and B-IV were 0 22 0 15 0 16 and 0 09 per cent respectively (Table 4 '0) No appreciable differences were noticed in the values of acetic acid in

frozen yogurt as compared to the corresponding values in set set yogurt

The content of acetic acid in frozen yogurt stored for 90 days did not change substantially The values after 90 days of storage under B-I B II B III and B IV were 0 21 0 09 0 15 and 0 06 respectively (Table 4 20)

5 16 Lactic acid - acetic acid ratio in set yogurt under different treatments

Homofermentative lactic acid bacteria ferment lactose to produce mainly lactic acid besides traces of other substances in milk The titratable acidity in the fermented milk products is often referred as lactic acid and express in terms of per cent equivalent lactic acid

Bifidobacterium bifidum are heterofermentative organisms and produce lactic acid and acetic acid in a molar ratio of 2 3 The titratable acidit in product containing bifidobacteria either as a single culture or in mixed culture with other lactic acid bacteria include lactic acid and acetic acid To calculate the ratio between lactic and acetic acid in the present experiment values of acetic acid were subtracted from the average values of titratable acidity in the corresponding treatment and thus the values of lactic acid were obtained Subsequently the ratios were calculated

The lactic acid acetic acid ratio in milk fermented with bifidobacteria was reported to be 2 3 (Scardovi 1986) Desjardins et al (1990) however reported wide species and strain variations in the concentration of acetic and lactic acid produced by bifidobacteria The ratio of lactic to acetic acid for different strains of B bifidum recorded by Desjardins et al (1990) were in the range of 1 0 4 to 1 1 4 Only five of the 19 strains of bifidobacteria had maintained the ratio of 2 3 between lactate and acetate in their investigation

Ventling and Mistry (1993) reported the variations in acetic acid lactic acid ratio due to concentration of protein in milk by ultrafilteration They found that the acetic to lactic acid ratio were in the range of 0 45 0 66 at different concentrations of protein in the mixes

The lactic acid acetic acid ratio in yogurt were reported to be important for the flavour characteristic of the product Tamime and Robinson (1988) found that the excessive acetic acid can adversely affect the typical flavour of the yogurt

The lactic acid acetic acid ratio under the treatments of part A in the present experiment was not

calculated as the content of acetic acid under these treatments was too low to be detected

The ratio found in set yogurt under B-I B-II B III and B IV were 0 27 0 17 0 19 and 0 09 respectively (Table 4 21) These ratio indicate that even though optimum growth of bifidobacteria were obtained under different treatments the concentration of lactic acid is more than that of acetic acid This suggest that these low concentration of acetic acid may not influence the organoleptic character of the product

Sensory characteristics of yogurt

5 17 Score for organoleptic characteristics of set yogurt under different treatments

The organoleptic characteristic of the set yogurt under different treatments were evaluated in terms of score obtained for general appearance body and texture flavour by a panel of five judges

The consumer s acceptability of any food prod icts is largely based on its flavour and texture but the consumers are first attracted by the appearance of the product The use of NDM for yogurt preparation is an established practice The condensed whey or whey protein dispersion may change the

general appearance and technological characteristic of the product The score for general appearance thus is a very important tool and may serve as a guide for assessing the consumer s response

The general appearance score under A I A II A III and A IV were 4 28 3 74 4 27 and 4 46 respectively out of maximum five ~~respectively~~ (Table 4 22a) These differences were found to be statistically non significant The general appearance score under A III was very close to control (A I) and the score was highest under A-IV It could be concluded that the use of condensed whey for replacement of 100 per cent NDM in yogurt did not adversely affect the general appearance and the fortification of whey protein dispersion in place of NDM improved the general appearance

Similar trend was observed for treatments under part B The score for general appearance under B I B II B III and B IV were 3 96 3 84 3 84 and 4 25 respectively On statistical analysis no significant differences were found between the treatments of part A with the corresponding treatment of part B

The body and texture score may indicate the quality of yogurt in terms of viscosity curd tension gel firmness and syneresis Higher levels of total milk solids in the mix are

reported be responsible for its physical properties especially the viscosity and gel firmness One of the objective of the present investigation is to assess the alteration in physical and technological properties of yogurt prepared from milk fortified with condensed whey or whey protein dispersion

The body and texture score under A I A II A III and A IV were 4 18 3 90 3 98 and 4 50 respectively (Table 4 22a) The score under A-IV was more than control (A-I) By this result it can be concluded that the body and texture score was significantly improved by the use of whey protein dispersion in yogurt mix The body and texture score of yogurt prepared by fortification of condensed whey were marginally lower than control Similar trend was observed under the treatments of part B The body and texture score under B I B II B III and B-IV were 4 05 3 83 3 95 and 4 20 respectively The body and texture score under the treatments of part B were slightly lower than the corresponding treatment of part A and differences between A I and B I A II and B II A III and B III and A IV and B IV were found to be statistically not significant (Table 4 22b)

The findings of the present experiment are in agreement with the result obtained by Broome et al (1982) Guirguis et al (1984) and Abd El Salam et al (1991) Experiment of Broome et al (1982) had proved that cheese whey

protein concentrate could replace 25 per cent of NDM in yogurt without affecting taste and textural quality Guirguis (1984) reported that fortification of yogurt pre mix with whey protein concentrate to partially replace the NDM improved viscosity characteristics and reduced yneresis Abd-El Salam (1991) found an increase in smoothness body and texture in yogurt fortified with cheese whey protein concentrate

Reduced syneresis and smooth texture of yogurt prepared with fortification of whey protein dispersion may be due to better water holding capacity of whey proteins (De wit 1989)

The flavour score of set yogurt under A I A II A III and A IV were 8 75 8 43 8 55 and 8 81 respectively (Table 4 22a) The values under A I and A IV were very close and that of A II A III were marginally lower than control (A I) Similar trend was also observed under the treatments of part B The flavour score under part B were 8 07 7 72 7 87 and 8 22 respectively The score under part B were slightly lower than the corresponding treatments of part A The reason for low flavour score under part B can be directly attributed to presence of acetic acid and lower concentration of diacetyl and acetaldehyde than that of treatments under part A The flavour score under treatment B II and B III were apparently lower than that of B IV

Lower flavour score obtained under B II and B-III as compared to that of B IV could be attributed to lower acetic acid and higher diacetyl concentration in latter The flavour score obtained under treatments of part A are in confirmation of the results obtained by Broome et al (1982) and Abd-El Salam (1990)

Marshall et al (1982) reported that the addition of ultrafiltered cheese whey protein and threonine into the milk and fermentation by a single culture of bifidobacteria produced a product similar to yogurt flavour

It can be concluded that the use of condensed whey has not affected the flavour characteristic of the yogurt substantially and the products fortified with whey protein dispersion are superior in flavour characteristics than that of control Further the supplementation of the starter culture of bifidobacteria along with traditional yogurt culture has not affected the flavour characteristic significantly

Total score under treatment A I A-II A III and A-IV were 17 23 16 11 16 36 and 17 80 respectively (Table 4 22a) Higher total scores under A IV were due to higher score for general appearance body and texture and flavour The total score under A-IV were significantly higher than A II and

A-III The corresponding total score under B I B II B III and B-IV were 16 17 15 55 15 38 and 16 67 respectively

From the score obtain for general appearance body and texture and flavour it can be inferred that condensed whey can be substituted for NDM in yogurt without affecting the organoleptic and technological characteristics The fortification of whey protein dispersion improved the flavour and body and texture characteristics of the set yogurt The total score under A IV and B IV were found to be highest The smooth texture of the yogurt due to presence of whey protein dispersion was appreciated by all the judges The higher water holding property of the whey proteins might have improved the textural properties of yogurt Thus it can be concluded that fortification of whey protein dispersion was found to be most suitable alternative for NDM in yogurt The product which was fortified with whey protein dispersion was found to superior in general appearance body and texture and flavour than the yogurt in which NDM was used

5 18 Score for flavour of frozen yogurt stored at -20°C for 0-90 days under different treatments

The sensory evaluation of frozen yogurt was carried out on the basis of score for flavour body and texture melting quality colour and package and bacteria

The score card adopted by American Dairy Science association for ice-cream (Nelson and Trout 1964) was used for evaluation of frozen yogurt

The flavour score of frozen yogurt immediately after freezing and hardening (0 day) under A-I A-II A III and A-IV were 42 17 40 83 41 63 and 42 43 respectively (Table 4 23a) No significant differences were found between the treatments The flavour score in yogurt fortified with condensed whey were slightly lower than control The flavour score of yogurt fortified with whey protein dispersion was very close to control (A I) Similar trend was observed under the treatments of part B No significant differences were recorded between treatment of part B with the corresponding treatments of part A It can be concluded that the fortification of condensed whey whey protein dispersion and inclusion of bifidobacteria as additional culture in yogurt did not affected the flavour of frozen yogurt

Contrary to the flavour score under part B of set yogurt the values of flavour score of part A and part B in frozen yogurt were very close Higher fat and sugar percentage in frozen product might have masked some of the undesirable flavour produce by acetic acid

The sensory evaluation of frozen yogurt was carried out at every 15 days interval till the end of experimental period to assess the quality of the product during storage

No substantial change in flavour score of frozen yogurt were noticed upto 90 days storage The differences in the score between 0 and 90 days under different treatment were statistically not significant except for B-II Under the treatment B-II even though a statistically significant difference was found between 0 and 90 day the decrease in the flavour score was very slight On 0 day the flavour score was 41 30 and it decreased to 40 57 on 90 day (Table 4 23a)

The result obtained by Mashayekh and Brown (1992) regarding the sensory evaluation ard flavour score in ice cream cultured with Streptococcus salivarius subsp thermophilus and L delbrueckii subsp bulgaricus supports the flavour characteristic obtained under present investigation

Opdahl and Baer (1991) conducted a consumer acceptance test of frozen yogurt which was fortified with cheese whey protein concentrate Their result indicated that the frozen yogurt was most accepted product in comparison with ice cream

Hekmat and McMohan (1992) conducted a sensory evaluation test of a probiotic ice cream The ice-cream mix was fermented by B bifidum and L acidophilus before freezing

and hardening They reported a favourable response for the flavour of the product from the judges

5 19 Score for body and texture of frozen yogurt stored at -20°C for 0-90 days under different treatments

The body and texture of the frozen yogurt was judged on a 30 point scale

The body and texture score of the frozen yogurt (0 day) under different treatments were in the range of 26 60 - 27 93 (Table 4 24a) The differences between the treatments were found to be statistically not significant (Table 4 24b)

This finding indicate that the body and texture of the frozen yogurt was not affected by the fortification of condensed whey or whey protein dispersion in place of NDM

The body and texture of a frozen dessert is reported to be entirely dependent on the size and distribution of ice crystals To maintain a characteristic fine texture for ice cream ice crystals should be very small angular and uniformly dispersed between air cells solidified fat globules and the unfrozen water phase (Keeney and Kroger 1987)

The composition of the mix markedly affect the size and shape of ice crystals The contert of fat and SNF in the mix are regarded as the most important ingredient which are responsible for physical structure of ice cream Raising of the fat and SNF to certain level favour small ice crystals both by mechanical obstruction and by the water binding property of extra protein

In the present experiment plain yogurt was used as a base for preparation of frozen yogurt As the yogurt contain 16 per cent milk total solids and five per cent sugar there was every chance of the formation of large ice crystals

The large ice crystals were not only responsible for sandiness in product but also detrimental to starter culture (Sheu et al 1993)

In order to avoid formation of large ice crystals the total solids levels of the yogurt were raised to 36 per cent by addition of cream and sugar after the fermentation and cooling The composition of frozen yogurt mix was adjusted as per the IS (1964) for the ice cream

Sodium alginate as stabilizer and GMS as emulsifier were used to maintain the physical characteristic

The largest single use of whey in a dairy product as reported by Mathur and Shahani (1979) was for the replacement of NDM in ice cream The code of federal regulations of USA allowed the use of cheddar cheese whey solids as a replacement of NDM in the ice-cream to a maximum limit of 25 per cent of the SNF in the mix

Opdahl and Baer (1991) reported that because there were no federal standard for frozen yogurt and no restriction on the amount of whey solids that can be added to frozen yogurt mixes its use to replace 100 NDM would be economically justified They found that the body texture and physical characteristics of the frozen yogurt was not affected by the incorporation of whey protein concentrate into the mix

Frozen yogurt being a product of recent origin and competing with the traditionally most popular dairy product ice cream (Knupp 1979 Kosikowski 1981) it is desirable that the body and texture of this product should be as perfect as that of ice cream

The composition of frozen yogurt used and the method of its preparation adopted in the pre ent experiment was found to be most suitable as judged by its technological characteristic body and texture

Different methods of preparation of frozen yogurt or cultured ice cream was reported in the literature

Laroia and Martin (1991) inoculated the mixed culture of thermophilus and bulgaricus at the rate of 1 25 per cent each into a ice cream mix The total solids content of the mix was 19 per cent After four hours of incubation at 43°C the desired pH of 3 6 4 6 was found After the fermentation the yogurt was pasteurized to destroy the starter culture The mix was then inoculated with combined culture of B bifidum and L acidophilus These starters were added 45 minutes before freezing After freezing and hardening the B bifidum count was $6\ 4x10^6$ and that of L acidophilus was $7\ 8x10^6$ cfu per ml

One of the limitation of the method adopted by Laroia and Martin (1991) was the destruction of thermophilus and bulgaricus Even though these two organisms are of non human origin they are found to have some therapeutic values Besides the final product will not be having the typical yogurt flavour as the volatile flavour are removed during the heat treatment Another disadvantage of heat treatment of fermented product is the syneresis The syneresis is considered as the serious defect in frozen products

Mashayekh and Brown (1992) prepared of frozen cultured ice cream with thermophilus and bulgaricus The ice-cream mix containing 40 per cent total solids was inoculated with one per cent inoculum of normal yogurt starter culture They claimed that the desired pH was reached within four to five hours at 43°C The mix was then frozen in ice cream freezer They found that smooth consistency in the product with 90 100 per cent over run

Hekmat and McMohan (1992) adopted a similar method for preparation of probiotic ice-cream with B bifidum and L acidophilus The rate of inoculum has four per cent for each organism The incubation was carried out at 42°C till the desired pH 4 9 attained It was reported that the desired pH was attained in five hours

5 20 Score for melting quality of frozen yogurt stored at -20°C for 0-90 days under different treatments

The melting quality score of frozen yogurt (0 day) under different treatments were in the range of 4 46 4 70 (Table 4 25a) The maximum point allotted for this characteristic was five

Arbuckle (1966) pointed out the melting quality of the ice cream was depended on mix composition emulsifier and stabilizers used Excessive stabilizers cause very slow

melting Very high fat content also was considered as one of the reason for slow melting Whey leakage from the ice-cream or frozen yogurt occurs during melting because of poor quality ingredient or improper balance of mix

Since these types of defects were not observed in the frozen yogurt prepared in the present experiment it can be concluded that the incorporation of whey solids in different forms had not affected the melting quality of frozen yogurt

The melting quality score during the period of storage did not change substantially After 90 days of storage of frozen yogurt the score were in the range of 4 17 4 53 No statistical significant difference was found in the score of melting quality between 0 and 90 days (Table 4 25b)

5 21 **Score for colour and package of frozen yogurt stored at -20°C for 0-90 days under different treatments**

The colour and package score for frozen yogurt (0 day) were in the range of 4 53 4 76 out of the maximum of five (Table 4 26a)

The colour and package of any product indicate among other things the sanitary and hygienic measures adopted during the preparation of dairy product

In the present investigation artificial colours were not used in frozen yogurt The colour and package score suggest that the condensed whey or whey protein dispersion can be used in the product without affecting its appearance The condensed whey contain high lactose Heating of supersaturated solution of lactose may cause browning and caramalisation due to interaction with proteins The yogurt mix under all treatments were heated to 80°C for 30 minutes before fermentation The colour and package scores for frozen yogurt obtained suggest no such defect like caramalisation It can be thus concluded that colour and packaging were not affected by the use of condensed whey or whey protein dispersion

The storing the frozen yogurt for 90 days had not resulted in any substantial change in the colour and package score After 90 days the score were in the range of 4 26-4 70

Comparison of colour and package score between 0 and 90 days indicate no significant differences under all the treatment except A I and B I Marginal but statistically significant reduction in the score was found after 90 days of storage of frozen yogurt under A-I and B I

Bacterial score

Bacterial score for frozen yogurt were not evaluated

and as such full score of 15 points were added to the total score of flavour body and texture melting quality and colour and package

The score for bacteria were included in the score card of ice cream so as to judge the presence of contaminants (Nelsen and Trout 1964) But as it was found difficult to evaluate the ice-cream for bacteria by the judges the full 15 points were usually allowed to be added into the total (Nelsen and Trout 1964) The similar procedure was adopted in the present experiment for the bacterial score for frozen yogurt

5 22 Total score of frozen yogurt stored at -20°C for 0 90 days under different treatments

The total score in frozen yogurt (0 days) under different treatments were in the range of 91 06 94 23 (Table 4 27a) Analysis of variance howed the differences between the treatments were not significant The score obtained for flavour body and texture melting quality and colour and appearance for the frozen yogurt indicate that a high quality set and frozen yogurt can be prepared by fortification of the mix with condensed whey Incorporation of whey protein dispersion in mix produced a superior product amongst all the treatments studied One of the most important advantage of fortification of whey solids is to reduce the

cost of production As it was found that whey solids both as a condensed form and as whey protein dispersion could replace the NDM in yogurt without affecting its sensory and technological qualities The utilization of whey solids in yogurt may also help in reduction of the problems of whey disposal

Frozen yogurt have an added advantage over the ice cream as it have a optimum level of viable starter bacteria The presence of viable starter culture ensure the therapeutic benefit of the product The frozen yogurt is found to be one of best vehicle for incorporation of bifidobacteria as a dietary adjunct The therapeutic and nutritional benefits of bifidobacteria and normal yogurt ulture were already described in the beginning

The total score of frozen yogurt after 90 days of storage was in the range of 89 60 91 86 (Table 4 27a) This also indicate that no substantial change in organoleptic characters of frozen yogurt had occurred during the storage

From the foregoing discussions it can therefore be concluded that

1 a good quality yogurt can be prepared by the fortification of different forms of whey solids as a substitute for NDM

thereby saving the cost of production and utilization of precious whey proteins

2 vacuum condensation of cottage cheese whey has proved to be a viable method for obtaining the whey solids in concentrated form The whey solids in this form can be used in set and frozen yogurt for improving their nutritive value

3 textural characteristics of set and frozen yogurt can be improved by incorporation of whey protein dispersion in place of NDM

4 yogurt can be one of the best vehicle for incorporation of B bifidum as a dietary adjunct as the optimum growth of B bifidum can be obtained when grown as a mixed culture with thermophilus and bulgaricus

5 eventhough the reduction in the count of the bulgricus was observed in yogurt when grown with bifidobacteria its growth can be improved by the incorporation of whey solids in place of NDM

6 conversion of yogurt into the frozen yogurt was found to be a good method for increasing the shelf life of the product without having a much deleterious effect on starter bacteria including bifidobacteria

Future prospects

From the present study it is proved that the condensed whey can be a better alternative to NDM in yogurt further studies on the following aspect may be useful in dairy industry

Studies can be undertaken to overcome the lactose crystalization during the freezing (because high lactose content in condensed whey) by hydrolysing the part of lactose in condensed whey with β-D-galactosidase enzymes Addition of whey solids in indigenous dairy products like Dahi and Rasogolla may improve their textural characteristics

Studies on the amino acid profiles in yogurt fortified with condensed whey solids and supplemented with bifidobacteria may be very useful for better understanding of the growth requirement of bifidobacteria and its symbiotic relationships with conventional yogurt starter bacteria

SUMMARY

SUMMARY

An experiment was undertaken to study the effect of incorporation of condensed cottage cheese whey and B bifidum in yogurt

Set and frozen yogurt were prepared with cow milk standardized to three per cent fat and 13 per cent SNF

Yogurt mixes were prepared with fortification of NDM condensed whey to replace 50 and 100 per cent NDM or with whey protein dispersion (as a complete substitute for NDM)

Streptococcus salivarius subsp thermophilus and L delbrueckii subsp bulgaricus were used as starter culture for fermentation of the yogurt mix under treatments of part A and B bifidum in combination with conventional yogurt culture for mix under treatments of part B

Set yogurt was used as a base for preparation of frozen yogurt

Microbiological analysis revealed that the incorporation of whey solids in different forms had stimulated the growth of thermophilus and bifidobacteria The bulgaricus count however remained stable in yogurt fortified with condensed whey

The growth of thermophilus was also stimulated by the incorporation of B bifidum as a supplementary culture however viable count of bulgaricus in yogurt supplemented with bifidobacteria were lesser in comparison to that of yogurt with only conventional starter bacteria, but growth of the bulgaricus can be improved by incorporation whey solids in the mix

Optimum growth of the bifidobacteria was recorded when grown in association with thermophilus and bulgaricus

The thermophilus bulgaricus and bifidobacteria count in set yogurt were in the range of 3 06 x 10^9 - 4 26 x 10^9, 2 36 x 10^9 - 3 91 x 10^9 and 2 25 x 10^9 - 3 48 x 10^9 cfu per ml respectively

The viable count of thermophilus bulgaricus, and bifidobacteria were reduced by less than one logarithmic unit during the process of freezing and hardening of yogurt Storing the frozen yogurt at 20°C for 90 days further reduced the count of starter bacteria by one to two logarithmic unit After 90 days of storage the viable count of thermophilus bulgaricus and bifidobacteria were in the range of 0 25 x 10^9 - 0 35 x 10^9 0 18 x 10^9 0 45 x 10^9 and 0 07 0 13 x 10^9 cfu per ml respectively These count were well above the dietary recommendation (10^6 10^7) suggested for beneficial therapeutic effects

From the biochemical studies it was found that the NPN in set yogurt varied from 46 69-63 03 mg per 100 g under different treatments

The tyrosine value were in the range of 0 17-0 24 mg per g in set yogurt under different treatments The highest tyrosine values were found in the products fortified with whey protein dispersion Gradual increase in tyrosine values in frozen yogurt were recorded during the period of storage After the end of 90 days these values were in the range of 0 27-0 28 mg per g

The concentration of diacetyl and acetaldehyde was affected both by fortification of whey solids and presence of bifidobacteria Their concentration ranged from 7 83-19 00 ppm and 31 50-43 04 ppm respectively in set yogurt under different treatments Highest concentration of diacetyl was found in yogurt fortified with whey protein dispersion while incorporation of condensed whey to substitute NDM (100 per cent replacement) produced highest level of acetaldehyde

The concentration of acetic acid in the product with conventional yogurt bacteria was too low to be detected by gas chromatograph The content of acetic acid in yogurt in which B bifidum was used as a supplementing culture was found to be in the range of 0 08 0 21 per cent No appreciable change in this concentration was found during the storage

Organoleptic evaluation suggested that the textural characteristic and flavour of yogurt could be improved by the incorporation of whey protein dispersion Presence of bifidobacteria had not affected the flavour score of yogurt significantly

The flavour body and texture of the frozen yogurt were not affected by the storage for 90 days Reduction in body and texture score and flavour scores were very marginal after the 90 days of storage

It can be concluded that the incorporation of whey solids was stimulatory to the growth of thermophilus and bifidobacteria Use of B bifidum as supplementary culture have increased the viable count of thermophilus however the growth of bulgaricus was adversely affected Yogurt was found to be an effective medium for incorporation of B bifidum as a dietary adjunct Eventhough there was a reduction in viable count of starter bacteria during the process of freezing and hardening and also during the frozen storage at -20°C still after the end of 90 days sufficient number of viable cells of thermophilus bulgaricus and bifidoba teria were present in the product so as to provide beneficial nutritive and therapeutic effect after its consumption

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EFFECT OF INCORPORATION OF CONDENSED CHEESE WHEY AND *Bifidobacterium bifidum* IN YOGURT

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ABSTRACT OF THESIS

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ABSTRACT

An experiment was conducted to assess the possibility of utilization of whey solids in different forms in yogurt as a substitute for NDM and also B bifidum as an adjunct with the view to improve the therapeutic value of yogurt

A detailed review of literature was presented on the morphological and physiological characteristics of starter cultures importance and utilization of whey solids effect of long term storage on starter bacteria and other related aspects

Methodology of condensation of cottage cheese whey preparation of whey protein dispersion and manufacture of set and frozen yogurt has been described Important analytical procedures were presented

The experiment comprised of part A and part B based on the starter culture The mix under the part A were fermented with conventional yogurt starter culture viz S salivarius subsp thermophilus and L delbrueckii subsp bulgaricus as against this the mix under part B were fermented with combination of conventional yogurt starter and B bifidum

Fa h part comprised of four treatments based on types of mill solids used to raise the content of SNF to 13 per

cent For mixes under A-I and B-I fortification was with NDM For A-II and B II condensed whey was used to replace 50 per cent NDM Mixes under A III and B III were fortified with condensed whey to replace 100 per cent NDM and whey protein dispersion was used to replace complete NDM under A-IV and B-IV

The results obtained had been compared with similar reported studies and conclusions were drawn

The data regarding the starter bacterial count indicated the optimum growth of thermophilus bulgaricus and bifidobacteria in yogurt fortified with different forms of whey solids The count of thermophilus and bifidobacteria were higher with the fortification of whey solids in yogurt mix and the growth of bulgaricus was not adversely affected in the presence of the whey solids

Incorporation of B bifidum stimulated the growth of thermophilus however it was found to have some inhibitory effect on bulgaricus count The inhibitory effect of B bifidum on bulgaricus was lesser in presence of whey solids than in yogurt fortified with NDM

Optimum growth of B bifidum was obtained when grown in association with conventional yogurt culture

The viable starter bacterial count was decreased by about one logarithmic unit during the process of freezing and hardening of yogurt at 20°C Additional reduction by one to two logarithmic units was also recorded during the frozen storage for 90 days At the end of 90 days of storage the population of viable starter bacteria however were higher than the minimum recommended range of 10^6-10^7 so as to provide the nutritional and therapeutic benefits after consumption

Coliform count in the set yogurt was in the range of 1 66 5 00 cfu per ml Yeast and mould count were 3 33 11 66 cfu per ml

Optimum acidity was produced in yogurt fortified with different forms of whey solids and also in presence of B bifidum The titratable acidity was in the range of 0 93 1 02 in set yogurt at pH 4 6 No appreciable change in titratable acidity and pH was observed during the storage of frozen yogurt

The content of NPN in set yogurt under different treatments were 46 69 63 03 mg per 100 g The tyrosine values were in the range of 0 17 0 24 mg per g There was a gradual increase in tyrosine value during the storage of frozen yogurt for 90 days at 20°C

The content of the diacetyl and acetaldehyde in the set yogurt were 7 83 19 00 ppm and 31 50-43 04 ppm respectively The yogurt fortified with whey protein dispersion contained highest concentration of diacetyl The concentration acetaldehyde in yogurt fortified with condensed whey as a substitute for complete NDM was significantly higher in comparison to products under other treatments

Incorporation of B bifidum as a supplementary culture reduced the levels of both diacetyl and acetaldehyde in set yogurt

The contents of acetic acid in set yogurt under the treatments of part B were 0 08-0 21 per cent and no acetic acid were detected in treatments under part B

Organoleptic evaluation indicated that the fortification of whey solids did not effected the flavour and textural characteristics in set and frozen yogurt On the contrary the flavour body and texture scores of set yogurt were improved by incorporation of whey protein dispersion

After 90 days of storage the organoleptic characteristics of the frozen yogurt has not changed appreciably

It can be concluded that a good quality set and frozen yogurt can be prepared with substitution of NDM by different forms whey of solids Amongst the different forms of whey solids the whey protein dispersion was found to be best From the present study it is also proved that the vacuum condensation can be one of the viable method for recovering whey solids from the byproducts of cotiage cheese

Yogurt can be a best vehicle for incorporation of bifidobacteria in a dietary adjunct Long time storage of frozen yogurt has not affected the microbiological in textural quality of frozen yogurt After the 90 days of storage of frozen yogurt at -20°C sufficient starter bacteria are present to have their beneficial therapeutic effect after consumption

Further studies on utilization of whey solids in indigenous dairy products may help in improving the nutritive value and reducing the whey disposal problems

Studies on amino acids profile of yogurt fortified with whey solids and supplemented with bifidobacteria may also prove beneficial in better understanding the growth requirements and symbiotic relationship between starter bacteria