STUDIES ON THE PREPARATION OF SYNBIOTIC YOGHURT-CHEESE FROM BUFFALO MILK

Thesis

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON THE PREPARATION OF SYNBIOTIC YOGHURT-CHEESE FROM BUFFALO MILK"** submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** with major in **Food Technology** and minor in **Process and Food Engineering** of the College of Post Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona fide* research carried out by **Ms. Pratima Khandelwal**, Id. No. **20860**, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

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CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Ms. Pratima Khandelwal**, Id. No. **20860**, a candidate for the degree of **Doctor of Philosophy** with major in **Food Technology** and minor in **Process and Food Engineering**, agree that the thesis titled **"STUDIES ON THE PREPARATION OF SYNBIOTIC YOGHURT-CHEESE FROM BUFFALO MILK"** may be submitted in partial fulfillment of the requirements for the degree.

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Introduction

1. Introduction

Globally India stands at top position in total fluid milk production with an annual production of 79.3 million metric tonne during the year 2000-01. Of the total quantity of milk produced, about 50-55 percent is transformed in to various milk products **(Mothey, 2001)**. Fermentation of milk has been practiced since time immemorial as means of transforming milk in to beneficial products bearing health-promoting attributes. The precise origin of fermented milk products could be 10-15 thousand years ago. Yoghurt is one of the widely used fermented milk product that was developed over thousands of years ago around the Mediterranean basin, Middle East and India **(Marshall, 1987)**.

Technically yoghurt is defined as a firm, creamy or liquid acidified milk product, which is manufactured from pasteurized milk using thermophilic lactic acid bacteria (*Lactobacillus delbrueckii* sub sp. *bulgaricus* and *Streptococcus salivarius* sub sp. *thermophilus)* which grow at an optimum temperature of 38-42^0 C. Yoghurt has been extremely successful in finding universal acceptance. The various forms of yoghurt available in the market are frozen yoghurt, dried yoghurt, smoked yoghurt, strained yoghurt, yoghurt butter, yoghurt-cheese apart from the flavored, low-calorie and carbonated types of yoghurt. The processes of producing variations in yoghurt essentially add to shelf life, novelty and do help in meeting specific demands of today's consumer. With

the rapid development of reduced fat and no fat products demanded by calorie conscious society, new growth areas for cultured milk products are opening. The fat and / or sugar are replaced by hydrocolloids, starches and functional dietary fibers in order to achieve desirable functional and quality attributes. Today, among cultured milk products, yoghurt is manufactured in many countries and fat/ sugar replaced yoghurt and yoghurt products are gaining market day by day **(Lo *et al.*, 1996).**

Yoghurt-cheese, also known elsewhere as labneh or concentrated yoghurt, is the product obtained by separating whey from yoghurt. **Kosikowski (1984)** mentioned that lactic acid coagulation of milk followed by contraction of the milk protein, which is collected together with fat, and other milk components retained in curd produced is called as yoghurt-cheese. He described yoghurt-cheese as a soft, acid, fresh variety resembling Neufachtel cheese. **Rasic (1987)** has reported that yoghurt-cheese is made as a fresh cheese with or without salt and is often flavored. Its processing is characterized by high heat treatment (80-85^{0}C/30min) of milk and specific type of yoghurt starters used for setting the cheese curd. As a result of concentration by whey removal, it has a consistency resembling cultured cream. Yoghurt-cheese should be soft, smooth, spreadable, not dry or grainy, having clean acidic flavor and milky white color. It is usually consumed with bread as a part of a main meal in some countries of Middle East **(El-Samragy and Zall, 1988; Hamad and Al-Sheikh, 1989; Tamime and Robinson, 1978, 1988)** and widely eaten

as a sandwich spread specially for breakfast and supper meals **(Dagher and Ali, 1985; Rao *et al.*, 1987)**. It also seems to be suitable vehicle for development of functional food variety.

A food is said to be functional if it contains component having positive effects or has physiological effect beyond traditional nutrient effect. Collectively, a functional food should have a relevant effect on the well-being and health or result in a reduction in disease risk. The development of functional foods is thus a unique opportunity to contribute to the improvement of the quality of food and consumer health and well being. Functional claims have already and will in the future lead to new concepts in nutrition. Examples of such new concepts are prebiotics and synbiotics, colonic foods and Bifidogenic factors **(Gibson and Roberfroid, 1995).**

Probiotics are technically defined as live microbial food ingredients that have a beneficial effect on human health. Some of the important beneficial effects are –antimicrobial activity, immune system modulation, antimutagenic activity, colonization resistance activity, maintenance of micro-ecology of bowel, stimulation of Bifidobacteria, deactivation of carcinogens etc. Commercially available probiotic strains belongs to genera *Lactobacilli, Bifidobacterium, Streptococcus, Bacillus, Bacteriodes, Pediococcus, Leuconostoc, Propionibacteruim* **(Mantera Alhonen, 1995)**, *Saccharomyces cerevisia* and *Aspergillus oryzae* **(Verma and Singh, 1995).**

Prebiotics are non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in colon, that can improve the host health **(Gibson and Roberfroid, 1995)**. The prebiotic approach advocates the administration of non-viable entities and therefore over comes survival problems in upper gastro-intestinal tract. Use of certain oligosaccharides that cannot be digested except through bacterial activity is a prebiotic approach **(Gibson, 1999).**

When prebiotics are used in combination with probiotics or live bacteria, the resultant has synergistic effects, referred to as 'synbiotics'. This is because in addition to the action of probiotics that promote the growth of existing strains of beneficial bacteria in the colon, prebiotics such as inulin and oligofructose also act to improve the survival, implantation and growth of newly added probiotics strains **(Niness, 1999).**

Inulin and oligofructose have attracted much attention recently as non-absorbable carbohydrates with prebiotic properties. Inulin (Raftiline) and oligofructose (Raftilose) are natural food ingredients commonly found in varying amounts in dietary foods. Inulin is a mixture of polymers consisting mainly of fructose unit; its partial enzymatic hydrolysis yields oligofructose. These are classified as prebiotic (dietary) fibers, which help in stabilization of foams, assist in digestion, increase stool volume, stimulate *Bifidobacteria* and are used for formulation of low fat preparations as fat and sugar replacers, respectively.

It is suggested that yoghurt-cheese could be a suitable vehicle to develop such functional foods while being a target for dairy research and development specialists. It bears good potential to be used in manufacture of low fat spread, low fat processed cheese, dressing and cheesecakes from milk. By incorporation of variations in terms of flavors, it could be easily used as dairy spread, high protein dietetic preparation. Such fermented dairy food with innovative use would feed the wide range of nutritional and organoleptic qualities being demanded by consumers of all ages, health status and cultures.

Very scanty information is available on incorporation of prebiotics and probiotics in yoghurt-cheese. Along with functional prebiotics, use of probiotic cultures for preparation of synbiotic yoghurt-cheese seems to be an effective area of research. In view of the above, the present investigation was undertaken to develop process for the preparation of synbiotic yoghurt-cheese from low fat buffalo milk.

Specific objectives of the study are as follows:

1. Optimization of the process parameters for the preparation of yoghurt-cheese
2. To study the effect of addition of prebiotic ingredients and enrichers on the physico-chemical and sensory attributes of yoghurt and yoghurt-cheese
3. To study the effect of using additional probiotic cultures on the physico-chemical and sensory attributes of yoghurt-cheese
4. To study the biocompatibility of starter-cultures
5. Evaluation of storage stability of yoghurt-cheese

Review of Literature

2. Review of Literature

Fermentation has been one of the oldest methods practiced by human beings for the transformation of milk to milk products with an extended shelf life and having special favorable organoleptic qualities. There are many different methods of carrying out fermentation in various parts of the world and these give rise to a range of fermented milk products including kumiss, kefir, acidophilus milk, yoghurt, *dahi*, cheese **(Berlin, 1962; Chandan *et al.*, 1969; Kosikowski, 1977; Tamime and Deeth, 1980).**

With the rapid development of reduced fat and no fat products as demanded by current calorie conscious consumers, new growth areas for cultured milk products have opened. The fat and/or sugar are removed and replacers hydrocolloids or starches are added to achieve the desirable organoleptic and rheological properties. Among the cultured milk products, yoghurt has been extremely successful in finding universal acceptance. Today it is manufactured in many countries and fat/ sugar replaced yoghurt and yoghurt products are gaining market day by day **(Lo *et al.*, 1996).**

2.1 Yoghurt

2.1.1 Definition

Yoghurt is the fermented milk product that has been developed over the thousands of years around the Mediterranean basin, the Middle East and India. The word 'yoghurt' is derived from the Turkish word 'juhurt'.

The product is known as laban or leben in lebenon, Iben in Morocco, juhurt in Turkey, zabadi in Egypt, dough in Iran **(Tamime and Deeth, 1980; Tamime and Robinson, 1988; Marshall, 1987).**

Yoghurt means a coagulated milk product obtained from toned milk or skim milk by lactic acid fermentation through the activity of *Lactobacillus delbrueckii* sub sp. *bulgaricus* and S*treptococcus salivarius* sub sp *thermophilus* **(Abrahamsen and Holmen, 1981; Kurmann, 1986).**

2.1.2 Yoghurt Ingredients

Cow's milk is generally used for the preparation of yoghurt, although buffalo's and goat's milk have also been used **(Kehagias *et al.*, 1986; Rysstad and Abrahmsen; 1987).** Yoghurt may also contain milk powder, skim milk powder, whey powder, whey proteins, caseinates and lactates enzyme preparation.

Sugar, corn syrup or glucose syrups may be used in sweetened and flavored yoghurt only. Fruits, fruit pulp, jam, fruit juice are added in fruit flavored or fruit yoghurt only.

Permitted stabilizers may be incorporated up to a maximum limit of 0.5 percent by weight. The titratable acidity of the product ranges from 0.8 to 1.2 percent as lactic acid, and pH from 4-0 to 4.5.

2.1.3 Proximate composition

The percentages of proximate composition in yoghurt prepared out of cow's skim milk are approximately moisture- 89-90, fat- 1.0, protein-3-3.5, lactose-5.0 and ash-0.7. The percent chemical composition of yoghurt (natural set and/or stirred) varied in total solids (TS) 12.0-18.7, fat 0.6-1.5, protein 4.5-6.4, lactose and other sugars 6.2-10.0 and ash 1.0-1.4 **(Tamime *et al.*, 1987).**

2.1.4 Types of yoghurt

The initial solid natural yoghurt has expanded in to many new products. The various types differ in chemical composition, their method of production, their flavor and the nature of post incubation processing. The yoghurt may be classified on the basis of following parameters-

2.1.4.1 Fat content

Three possible types of yoghurts classified according to fat content are full, medium or low fat yoghurt. This classification is useful in the formulations to facilitate standardization of product and to protect the consumers **(FAO/WHO, 1973).**

2.1.4.2 Method of production and physical structure of the coagulum

Under this category, there are two main types- set and stirred yoghurt.

- Set yoghurt is the product when fermentation is carried out in retail container and the yoghurt is in a continuous semi-solid mass.

- Stirred yoghurt is produced when coagulum is prepared in bulk and the gel structure is broken before cooling and packaging.

2.1.4.3 Flavoring of yoghurt

Flavoring divides yoghurt in to three categories -

- Plain or natural yoghurt is the traditional yoghurt with its sharp and 'nutty' flavor.

- Fruit yoghurt is made by addition of fruits (as preserves, puree or jam).

- Flavored yoghurt is prepared by adding sugar or other sweetening agents and synthetic flavorings and colorings to plain or natural yoghurt.

2.1.4.4. Post incubation processing of yoghurt

The emergence of new yoghurt products has been largely due to recent developments in this field. Some main examples are-

- **Pasteurized/UHT Yoghurt**

This is heat-treated yoghurt, leading to destruction of starter bacteria and reduction in the level of volatile compounds associated with flavor **(Schulz and Voss, 1970; Klupsch 1980)**. It increases the shelf life from 3 weeks to 6-8

weeks at 12^{0}C by heating at 60-65^{0}C. Generally, yoghurt is subjected to flash treatment at 60-70^{0}C after incubation **(Klupsch, 1972).**

- **Concentrated/ condensed yoghurt**

Partial separation of the liquid phase from yoghurt leads to the production of concentrated yoghurt of around 24 percent total solids with rheological properties and characteristics considerable different from those normally associated with yoghurt **(Tamime, 1987; Robinson and Tamime, 1975).**

- **Frozen yoghurt**

It is a product whose physical state resembles ice cream. It requires higher quantities of sugar and stabilizers to maintain the air bubble structure during freezing. It may be soft or hard.

- **Dried yoghurt**

It may be produced by sun drying, spray drying or freeze-drying. The process causes loss of some flavor compounds.

- **Carbonated yoghurt**

Duitschaener and Ketcheson (1974) developed a product called ' yoghurt-beverage' by carbonation of yoghurt. Carbonation improved the thirst-

quenching quality and enhanced its refreshing character. It involved post-production homogenization at a pressure of 175 kg/cm^2.

2.1.4.4Low Calorie yoghurt

The low calorie yoghurt is a favor among the diet conscious consumer, the energy content is reduced by adding stabilizes and thickening agents to boots the viscosity. The percent composition of a typical low-calorie yoghurt is milk SNF 9, fat 1, and stabilizer 0.5-1.0 (e.g. carrageenan: gelatin in 1:1). It has calorific value of 170 kJ/100g against 420 kJ/100g of plain yoghurt.

2.1.4.5 ß-D-galactosidase treated yoghurt

Hydrolysis of lactose is achieved by using ß-D galactosidase and this produces low- lactose-yoghurt. Hydrolysis of lactose leads to sweeter product **(Tamime and Deeth, 1980).**

2.1.4.6 Use of preservatives and gas flushing

Use of preservatives is prohibited in the yoghurt industry, although use of fruits in flavor yoghurt may carry preservatives (sorbic acid, its salts, SO_2, and benzoic acid @ 50 mg/kg in the final product) **(FAO/WHO, 1976).** Preservatives at 0.1 percent (w/v) in yoghurt can effect growth of starter culture and cause acetaldehyde levels to decrease more rapidly during storage **(Hamdan *et al.*, 1971; Nakae *et al.* 1971).** Alternatively, preservation of fruit flavored yoghurt is achieved by CO_2 or N_2 flushing. Flushing restricts growth

of yeasts and molds in the product. Combination of post incubation heat treatment with flushing improved the keeping quality of yoghurt for more than 4 weeks at 15^0C **(Klupsh, 1969a; 1969b).**

2.1.5 Manufacture of Yoghurt

2.1.5.1 Standardization and fortification of milk

The fat content in milk varies according to market demands and legal standards. The methods employed for the standardization of milk fat are removal of fat from full cream milk, mixing full cream milk with skim milk and addition of cream to skim milk. Fortification of milk with solids-not-fat (SNF) is synonymous with standardization of the SNF in yoghurt milk, i.e., fortification with lactose, ash and protein. The total solids in the milk are increased to a level that imparts desirable consistency and aroma to the yoghurt. In general, an increase in total solids will enhance these properties. The Total solids vary from as low as 9 percent in skim milk to over 20 percent in other types of yoghurt **(Tamime and Deeth, 1980).** The review by **Robinson and Tamime (1978)** showed that the recommended range of total solids in milk for yoghurt preparation is 14-18 percent. The desired SNF content in the yoghurt could be achieved by addition of milk powder (skim milk powder, whey powder or caseinates), concentration of milk by way of evaporation, ultrafiltration or reverse-osmosis or addition of miscellaneous products such as soybean protein and leaf protein isolates.

At this stage of processing, other derived ingredients added to yoghurt milk are sugar and stabilizers. Sugar is added to sweeten and the recommended level is governed by consumers' preference. Hydrophilic stabilizers such as starch, gelatin, guar gum, alginate, carrageenan or combinations thereof, are added in order to improve the viscosity of the product and to prevent whey syneresis. The maximum limit of stabilizer in yoghurt milk is 0.3 present. Higher levels can increase consistency, but the flavor gets adversely affected **(Tamime and Robinson, 1988).**

2.1.5.2 Homogenization

Homogenization of yoghurt milk is carried out at 9.8-19.6 MPa (100-200 kg/cm^2) at 50-60^{0}C. It prevents the tendency of fat rise to the surface. It reduces the synersis due to protein-protein interaction; this improves the water holding capacity of the coagulum. It reduces the tendency of small fat globules to coalesce and improves light scattering of the product, making it look whiter. Homogenized milk has tendency to foam due to increased levels of phospholipids in the skim phase **(Tamime and Robinson, 1988).**

2.1.5.3 Heat treatment

The application of heat to yoghurt milk is a universal practice and the reported time-temperature treatments can be classified as 85^{0}C/30-45 minutes, 90-95^{0}C/5-10 minutes, 115^{0}C/30 sec or 149^{0}C/3.3 sec. The most important heat induced changes in relation to yoghurt manufacture are changes in physico-

chemical structure of proteins, the lowering of the pH, destruction of undesirable micro-organisms in milk, production of compounds which stimulate *Lactobacillus delbrueckii sub sp. bulgaricus*, production of volatile compounds which contribute to the flavor, changes in ionic status of minerals and changes in physico-chemical nature of the nitrogenous components of the milk. The heat treatment (85^0C/30-45 min or 90-95^0C/5-10 minutes) is sufficient to denature whey proteins and induce interactions between k-casein, β-lacto-globulin and possibly α-lactalbumin. Heating also increases hydrophilic properties of the coagulum and the stability of yoghurt **(Ling, 1963; Tamime and Deeth, 1980).**

2.1.5.4 Fermentation

Invariably, *Lactobacillus delbrueckii* sub sp. *bulgaricus* and *Streptococcus salivarius* sub sp. *thermophilus* are used as yoghurt starters, @ 2-3 percent in 1:1 ratio. The cultures being thermophilic, incubation is carried out at 42-45 ^{0}C for 3-4 hours.

2.1.5.5 Changes in milk during fermentation

The marked changes are produced on carbohydrates, protein, fat mineral, and vitamin components along with production of flavor compounds, particularly acetaldehyde, some enzymes and bacterial mass.

2.1.5.5.1 Carbohydrates

Lactose is broken into glucose and galactose by β-galactosidase produced by starter culture. The glucose is rapidly metabolized into lactic acid. Some 3 percent lactose is converted giving 1.5 percent galactose and 1 percent lactic acid. Most yoghurt can he expected to have about 5 percent lactose **(Deeth, 1984)**. *S. thermophilus* uses only the glucose, part of lactose and D-galactose is excreted in to the product **(Teuber, 1995)**. Lactic acid in cultured products gives the typical sourness and refreshing taste. It acts as a preservative and causes milk to clot. Yoghurt contains equal amount of both isomers of lactic acid, D (-) and L (+). The final lactic acid concentration is around 1.2 percent **(Teuber, 1995)**. The L (+) isomer is rapidly metabolized where as D(-) isomer gets slowly metabolized. *Bifidobacteria* based fermented milk products contains some 90 percent desired L (+) lactic acid **(Klupsh, 1983)**.

The level of D (-) lactic acid in yoghurt increase during storage due to activity of *L. delbrueckii* sub sp. *bulgaricus,* which is more acid tolerant than *S. salivarius* sub sp. *thermophilus* **(Abrahamsen, 1978).**

2.1.5.5.2 Proteins

Protein in yoghurt is generally considered to be more digestible than that of milk due to nature of protein curd, partial degradation and changes due to heating. Proteolytic activity has been demonstrated by yoghurt cultures **(Sarkar and Misra, 1994)** and *B. bifidum* **(Misra and Kuila, 1991)**. Proteases

and peptidases are produced by bacteria act on milk proteins, resulting in increase of peptides and free amino acids. The soluble non-protein nitrogen content in yoghurt is about 50 percent higher than that in original milk mix **(Deeth, 1984).**

2.1.5.5.3 Fats

The lipolytic enzymes mainly lipases produced by starter bacteria cause lipolysis. These lipases usually show a preference for short chain triglycerides and have been reported to show higher activity against partial glycerides than against triglycerides **(Stadhouders and Veringa, 1973).** An increase in volatile fatty acids has also been reported during fermentation and storage of yoghurt **(Formisano *et al.*, 1971).**

2.1.5.5.4 Vitamin and mineral

During heating, some of the vitamins B_6, B_{12} and C and folic acid are partially destroyed. Vitamin B_{12} is utilized by the starter bacteria during fermentation. Synthesis of niacin, folic acid **(Reddy *et a.*, 1976)** and B_{12} **(Kaneko *et al.*, 1986)** by yoghurt culture and vitamin B_1, B_6 and folic acid by *B. bifidum* **(Hamada, 1966)** takes place. The variation in manufacturing method, content of additives such as fruit and stabilizers and conditions of storage dictates the actual levels of vitamin in product **(Deeth, 1984).**

Yoghurt is also rich in mineral content. Calcium and phosphorus are more available from yoghurt than milk and their absorption in the gut is facilitated by lactic acid.

2.1.5.5.5 Production of antibacterial substances

There have been several reports of the production of antibacterial substances by the starter bacteria used in the preparation of cultured dairy products. *L. delbrueckii* sub sp. *bulgaricus* and *S. salivarius* sub sp. *thermophilus* have been shown to produce antibacterial substances in milk **(Anand *et al.* 1984; Sarkar and Mishra, 1996; Stefanova, 1979)**. Types, mechanism of action and proposed positive health benefits of antibacterial substances have been discussed under 'probiotics' section.

2.1.5.5.6 Production of other compounds

L. delbrueckii sub sp. *bulgaricus* is solely responsible for production of 20-40 mg benzoic acid/Kg **(Teuber, 1995)**. Hydrogen-per-oxide, the product of oxygen metabolism of starters, is accumulated @ 10 mg/kg **(Condon, 1987).**

2.1.5.6 Cooling

Cooling checks the metabolic activity of the lactic acid bacteria, controls the acidity of the product and initiates cold gelation of the curd. The most suitable moment to begin cooling is when the pH is 4.7-4.5. Too slow and too

fast cooling causes super maturation of yoghurt and contraction of curd, respectively **(Tamime and Robinson, 1988)**.

2.1.6 New yoghurt products

The consumer appeal for yoghurt has broadened. It has been attempted to introduce variety of yoghurt products in the market. Some of these products are yoghurt with added cream, yoghurt free from additives such as artificial colors, stabilizers and sugar, yoghurt mousse/lightly whipped yoghurt (blend of yoghurt, cream and fruit puree) and use of fructose in fruit flavored preparations, aspartame, acesulfame-K in dietetic yoghurt preparations **(Tamime and Robison, 1988)**. Therapeutic products of yoghurt are becoming popular with health conscious consumers, which are produced by incorporation of thermophilic microflora such as *Bifidobacterium* spp. (isolated from newly born babies) and L. acidophilus (which can establish in gut) **(Rasic and Kurmann, 1978)**. The products are branded as Bioghurt, Biograde, and Bifighurt.

2.1.7 Yoghurt related products

2.1.7.1 Labneh and strained/concentrated yoghurt

These products are popular in Middle East and neighboring countries. The natural yoghurt is strained using a cloth bag, animal skin or earthenware vessel. The product is then known as labneh or lebneh (in Lebanon, Arab

countries), tan or than (in Armenia), tulum (in Turkey) and lebnen zeer (in Egypt).

Factory scale production is accomplished by using a modified nozzle separator **(Tamime and Robinson, 1988). Dagher** and **Ali (1985)** produced labneh from heated yoghurt by centrifugation for 5 min at different speeds (4080, 7970, 11700 g). Detailed review in presented under section yoghurt-cheese / labneh.

2.1.7.2 Chakka and shrikhand

This is an Indian type fermented milk product like labneh. Invariably, chakka is made from skimmed buffalo milk. Different starters have been employed and amongst them, LF-40 has been accepted as most suitable. After fermentation, the product is concentrated using a basket centrifuge at 900g for 90 min. Shrikhand is produced by blending chakka with cream sugar and flavors. The percent composition of shrikhand is as follows: total solids 57-60, fat 5-6, protein 6.5-7.0 sucrose 40-43, reducing sugar 1.6-1.7, ash 0.49-0.55, and titratable acidity 1.05-1.1 lactic acid **(Patel and Abd-Salam, 1986)**.

2.1.7.3 Shyr

Shyr is an Icelandic concentrated and fermented milk product. The percent chemical composition is around total solids 18.5-20.8, fat 0.30, protein 13-15, lactose 3.5, ash 0.7-1.0 and titratable acidity 1.86-2.72 lactic acid. The microflora present in skyr consists of *L. delbrueckii* sub sp. *bulgaricus*, *S.*

salivarius sub sp. *thermophilus, L.jugurti and L. helviticus* and lactose fermenting yeast **(Petursson, 1949).**

2.1.7.4 Labneh anbaris

It is a cheese like product, which has twice the level of milk solids of labneh and is preserved in olive oil. A typical product should contain fat 20 **(Rosenthal *et al.*1980)**, Total solids 48, protein 17.6, lactose 4.0 and ash 5 percent **(Tamime and Robinson, 1988).**

2.1.7.5 Kishk

It is a blend of yoghurt or other related products (labneh and/or laban zeer) and parboiled cracked wheat (burghol). The mixture is boiled, concentrated, shaped in to rolls of 10 cm in diameter and then dried in sun. The dried product is non-hygroscopic having a shelf life up to 2-3 years **(Abou-Donia 1984).**

2.1.7.6 Madeer or Oggtt

It is a product similar to kishk, made with out added cereals. The dried product is reconstituted and drunk like yoghurt. It is a product of Saudi Arabia and is prepared from goat's and ewe' milk **(Tamime and Robinson, 1988).**

2.1.8 Yoghurt as a vehicle for probiotics

2.1.8.1 Probiotics

Probiotics are the products bearing beneficial live microflora. Technically, probiotics are defined as live microbial food ingredients that have a beneficial effect on human health **(Salminen *et al.*, 1998)**.

It was Parker who coined the word probiotic in 1974, which means 'for life' or 'supporting life'. The concept of probiotics evolved around the turn of the 20th century when Elie Metchnikoff suggested that the long healthy life of Bulgarain peasants resulted from consumption of fermented products **(Bibel, 1998; Sanders 2000)**.

The probiotic bacteria most commonly studied include members of genera *Lactobacillus, Bifidobacterium, Saccharomyces boulardii* **(McFarland *et al.*, 1994)**, *Escherichia coli* **(Kruis *et al., 1997)***. Enterococcal strains have been as probiotics in non-food products.

2.1.8.2 Probiotic starters in yoghurt

Conventionally, yoghurt should have microflora consisting of *L. delbrueckii* sub sp. *bulgaricus and S. salivarius* sub sp. *thermophilus*. However, recent entrants in market contain *L. acidophilus, L. casei* and *Bifidobacterium*. These cultures are probiotic in nature. Such and its derived products are known as Bioghurt®, Biograde® and Bifihurt® **(Tamime and Robinson, 1988)**. The word 'bio' means healthy and natural **(Driesson and deBore, 1989)**.

Yoghurt starters are bile sensitive, where as the probiotic *lactobacilli* and *Bifidobacteria* are generally bile resistant/tolerant **(Sanders, 2000)**. Among the latter cultures, *L. acidophilus* is homo-fermentative and ferments lactose to DL-lactic acid where as *Bifidobacteria* are hetero-fermentative and ferment lactose to L (+) lactic acid and acetic acid in the ratio of 2:3 **(Driesson and deBoer, 1989)**. Yoghurt has become a popular vehicle for incorporation of probiotic *L.acidophilus* **(Hull *et al.*, 1984)** and *B. bifidum* **(Holecomb *et al.*, 1991)**.

2.1.8.3 Commercial application of probiotic cultures

Probiotics could be combined with other healthful ingredients such as dairy foods or simply used to complement the natural functional attributes of whole foods. Such products have added benefit of enhancing consumer nutrition. A safe, cost effective, natural barrier to microbial infection or to the negative effect of indigenous microflora may be significant to human health **(Sanders, 2000).**

The commercial use of probiotics, however, has proceeded even though complete mechanisms of action are yet to be explored. This is because essentially no risk is associated with consumption of well-defined probiotics in food and many benefits are possible. Perhaps the most compelling evidence for probiotic efficacy is in the areas of anti diarrheal effects and improved digestion of lactose in lactose intolerant people, because their findings have

been sustained in human studies and in more than one laboratory **(Sanders, 1999)**.

Commercially available probiotic strains belongs to genera *Lactobacilli, Bifidobacterium, Streptococcus, Bacillus, Bacteriodes, Pediococcus, Leuconostoc, Propionibacteruim* **(Mantera Alhonen, 1995),** *Saccharomyces cerevisia* and *Aspergillus oryzae* **(Verma and Singh, 1995).**

2.1.8.4 Effects on human health

Probiotic bacteria may mediate a variety of health effects through numerous proposed mechanisms. Probiotics have been suggested to play a role in variety of health effects and mechanism for probiotic functionality is as follows **(Sanders, 2000)**-

- Anti microbial activity
- Immune effects
    a. Adjuvant effect
    b. Cytokine expression
    c. Stimulation of phagocytosis by peripheral blood leucocytes
    d. Secretory Ig A
- Antimutagenic effects
- Antigenotoxic effects
- Influence an enzyme activity
- Enzyme delivery

The sum of processes by which probiotics inhibit colonization of other strains is called colonization resistance. Much work remains to classify the mechanisms of action of particular probiotics against particular pathogen. In addition, same probiotic may inhibit different pathogen by different mechanisms. The common mechanism by which probiotics may protect the host against intestinal diseases are-

- Production of inhibitory substances - Organic acids, hydrogen-peroxide, bacteriocins produced reduce the number of viable cells, affect bacteria (both Gram (-) and Gram (+)) metabolism **(Rolfe, 2000).**

- Blocking of adhesion sites - Competitive inhibition for bacterial adhesion sites on the intestinal epithelial surface occur **(Kleeman & Klaenhammer, 1982; Goldin *et al.*, 1992).**

- Competition for nutrients- probiotics utilize the nutrients otherwise consumed by pathogens, thus compete with latter. In vivo evidence is still lacking **(Rolfe, 2000).**

- Degradation of toxin acceptor- the postulated mechanism by which *S. boulardii* protects animals against *C. difficile* intestinal disease is through degradation of toxin receptor on intestinal mucosa **(Pothoulakis et al., 1993).**

Stimulation of immunity- both specific and non-specific immunity is suggested in order to protect intestinal disease **(Perdigon and Alvarez, 1992; Kalia *et al.*, 1992; Fukushima** et al., **1998).**

2.1.8.4.1 Potential and established effects of probiotic bacteria

Probiotic bacteria may mediate a variety of health effects through numerous proposed mechanism (Table 2.1).

Probiotic bacteria differ on the basis of genus, species and even strains. The strains of some species could be expected to differ in traits such as stability expression of enzymes, extent and types of inhibitors produced, carbohydrate fermentation patterns, acid producing ability, resistance to acid and bile, ability to colonize the gastro-intestinal tract and perhaps most importantly clinical efficacy. This microbiological circumstance does impose a burden of proof upon those attempting to commercialize probiotic bacteria **(Sanders, 2000).**

Some other health effects have been seen where probiotics mediated reduction in the severity of reaction to exposure to radioactive isotopes has been show in humans **(Henriksson *et al.*, 1995, Korshunov *et al.*, 1996; Salminen *et al.*, 1988).** Probiotics may also improve animal agriculture through greater resistance of farm animals to infection diseases **(Fuller, 1998).**

Table 2.1 Potential and established effects of probiotic bacteria

Target Health Benefit	Postulated Mechanism
Aid in lactose digestion	Bacterial lactase hydrolyses lactose
Resistance to enteric pathogens	Secretary immune effect
	Colonization resistance
	Alteration of intestinal conditions to be less favorable for pathogen city (pH, short chain fatty acids, bacteriocins)
	Alteration of toxin binding sites
	Influence on gut flora populations
	Adherence to intestinal mucosa, interfering with pathogen adherence
	Up regulation of intestinal mucin production, interfering with pathogen attachment to intestinal epithelial cells
Anti colon cancer effects	Mutagen binding
	Carcinogen deactivation
	Inhibition of carcinogen producing enzymes of colonic microbes
	Immune response
	Influence on secondary bile salt concentration
Small bowel bacterial growth	Influence on activity of over growth flora, decreasing toxic metabolism production
	Alteration of intestinal conditions to be less favorable to over growth flora activities or populations
Immune system modulation	Strengthening on non-specific defence against infection and tumor
	Adjuvant effect in antigen-specific translocation in to blood stream
Allergy	Prevention of antigen translocation in to blood stream
Anti hypersensitive effect	Peptidase action on milk protein yields tripeptides which inhibit angiotension 1 converting enzyme
Urogenital infections	Adhesion to urinary and vaginal tract cells
	Colonization resistance
	Inhibitors production
Infection caused by *Helicobacter pyroli*	Production of inhibitors of *H. pyroli* (lactic acid and others)
Hepatic encephalopathy	Inhibition of urease –producing gut flora

(Adapted from Sanders and Huis in't Veld, 1999)

2.2 Yoghurt-cheese or Labneh

One of the popular cultured dairy products in the Middle East is concentrated yoghurt, strained yoghurt, super yoghurt, yoghurt-cheese or 'Labneh' **(Gilles and Lawrence 1981; Kjaergaard-Jensen and Nielson, 1982; Tamime and Robinson 1978, 1988).**

2.2.1 Definition

Labneh is defined as semi-solid product obtained from yoghurt after removal of part of water, lactose and salt **(El-Samragy, 1997).**

Kosikowski (1984) mentioned that lactic acid coagulation of milk followed by contraction of the milk protein, which is collected together with fat, and other milk components retained in curd produced is called as yoghurt-cheese. He described yoghurt-cheese as a soft, acid, fresh variety resembling Neufachtel cheese. **Rasic (1987)** reported that yoghurt-cheese is made as a fresh cheese with or without salt and is often flavored. Its processing is characterized by high heat treatment (80-85^{0}C/30min) of milk and type of bacteria (yoghurt starters) used for setting the cheese curd.

2.2.2 Composition

The chemical compositions as well as sensory properties of labneh differ according to the type of milk used in its preparation **(El- Samray, 1997).** The suitability of using different kinds of milk i.e., dried whole milk, recombined

milk or butter milk, cow's or buffalo's milk has been investigated. **Bayer and Mair-Waldburg (1974)** reported that almost any dried whole milk of good organoleptic quality could be used for making labneh.

Tamine (1978) reported that the concentrated yoghurt manufactured from hydrolyzed milk was sweeter and more acceptable and its shelf life could be prolonged if the product was heat treated and packed aseptically. UF recombined skim milk powder and anhydrous milk fat was used successfully for labneh manufacture **(Abd-Salam and El-Alamy, 1982; Chehade *et al.*, 1992)**. Use of cows milk in Israel **(Rothenthal *et al.*, 1980), in U.K. (Tamime *et al.*, 1989; 1991),** in U.S. **(El-Samragy and Zall, 1988; Rao *et al.*, 1987)**; goat's milk in U.S. **(Rao *et al.*, 1987)**, in U.K. **(Tamimie *et al.*, 1991)** and sheep's milk in U.K. **(Tamime *et al.*, 1991)** for labneh manufacture have been reported. Full fat buffaloes milk **(El-Samragy *et al.*, 1990)** and butter milk **(El-Samragy *et al.*, 1988)** were used for labneh in Egypt.

Whey protein concentrate (WPC) was mixed with UF milk retentate (TS 22 %) at levels of 5, 10 and 15 percent for labneh making using different heat treatments (80^0C/15 min and 80^0C/20 min) **(Mahfouz *et al.*, 1992)**. Use of WPC affected the chemical composition slightly, improved the texture, reduced wheying off and had an acceptable quality. Use of vegetable oil (corn, soy, palm, sunflower) had no improvement either on composition or acceptability **(El-Samragy, 1997).**

Use of different starter cultures for the manufacture of labneh is responsible for variation in its composition and quality **(El-Samragy *et al.*, 1988; Rasic *et al.*, 1987; Rothenthal *et al.*, 1980; Tamime and Robinson, 1978; Tamime *et al.*, 1989). Osman *et al.*, (1992)** mentioned that for good labneh, a starter culture consisting of *Streptococcus lactis, S. cremoris, S. diacetylactis* and *L cremoris* should be used @ 2 percent (w/v) with incubation temperature of 32^0C. **Abou-Donia *et al.*, (1992)** assessed the chemical, microbiological and organoleptic characteristics of different preparation of labneh manufactured using nine different combinations of lactic starter cultures. **Ibrahim *et al.*, (1994)** attempted direct acidification of fresh buffaloes milk using lactic acid to pH values of 5.0, 4.8 and 4.6 to manufacture labneh.

2.2.3 General Characteristics

As a result of yoghurt concentration, labneh has a consistency resembling cultured cream. It should be soft, smooth, spreadable, not dry or grainy, having acidic clean flavor and milky white color. There should be no sign of wheying off **(El-Samragy and Zall, 1988; Hamad and Al-Sheikh, 1989)**. It is produced by strains of thermophilic lactic acid bacteria i.e. *Lactobacillus delbrueckii sub sp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus* which ferment lactose to produce a variety of organic acids mainly lactic acid. The percent composition may vary with in the range fat 9-11, carbohydrate 3.5-4, protein 8.5-9,Total solids 22-26, lactic acid 1.6-2.5, and salts about 1-1.5 **(El-Samragy, 1997)**. The addition of some stabilizers

such as starch and CMS @ 1.0 and 0.4 percent respectively improved the acceptability of labneh **(Dagher and Ali, 1985).**

2.2.4 Uses

It is usually consumed with bread as a part of a main meal in some countries of Middle East **(El-Samragy and Zall, 1988; Hamad and Al-Sheikh, 1989; Tamime and Robinson, 1978, 1985)** and widely eaten as a sandwich spread specially for breakfast and supper meals **(Dagher and Ali, 1985; Rao *et al.*, 1987).** Also, it is served with adder salt, olive oil and scooped with a piece of bread **(Basson, 1981).**

The properties of labneh and its manufacture classified it as a fresh, soft, unripened cheese specified as a yoghurt-cheese **(El-Samragy, 1997; Tamime, 1987).** It has been suggested that development of new uses for labneh/yoghurt-cheese or new dairy products originating from yoghurt-cheese should be a target for the dairy research and development specialists. Such product could be used as a dairy food in manufacture of low-fat processed cheese, low fat cheese sauce, dressing or topping and cheesecakes. These fermented dairy foods and their innovative use will meet a wide range of nutritional and organoleptic qualities being demanded by consumers of all ages, health status and cultures **(El-Samragy, 1997).** With the emergence of a strong preference for 'functional foods' among the calorie conscious consumers, yoghurt-cheese could also be a suitable vehicle to develop newer designed dairy food(s).

2.2.5 Traditional process of manufacture

Labneh has been traditionally home made in the Middle Eastern countries such as Egypt, Jordan, Israel, and Lebanon etc. It required considerable time, labour, regulated temperature and sufficient hygienic conditions for whey drainage and curd handling **(Abd-El-Salam and El-Alamy, 1982, El-Samragy and Zall, 1988 and Tamime and Robinson, 1985)**. The conventional method for producing labneh is to make yoghurt and store it overnight under refrigeration. On next day, salt is added and mixed thoroughly; it is then put in to cheesecloth bags and hung to drain for 12-24 hours, patched and stored under refrigeration **(El-Samragy *et al.*, 1988, 1990; Hefnny *et al.*, 1992).**

2.2.6 Modifications in traditional process of manufacture

Some suggested methods to hasten the conventional method of manufacture are cutting of coagulum of the set yoghurt, placing in cloth bags and applying a light pressing, using special centrifuge for whey separation and using ultra filtered retentate where milk is concentrated to the desired level of TS in labneh before fermentation **(Rasic, 1987).** All these approaches help to overcome sanitary problems usually caused by using cloth bags **(Tamime and Robinson, 1978).**

The use of whole milk powder with a reduced lactose content, reconstituted up to 30 percent Total solids, avoids the need for a drainage step

is of considerable interest **(Gilles and Lawrence, 1981)**. Increase of total solids in milk to 20, 24and 25 percent decreased whey drainage time to 10, 8 and 6 hours, respectively. Also, the product exhibited an improvement in processing and quality characteristics with a high acceptability **(El-Samragy *et al.*, 1990)**.

An attempt to modify the manufacturing process of labneh has been made by **Abou-Donia *et al.* (1992)**. They used different heat treatments i.e., 85^0C/15 sec or 85^0C/15 min, with or with out steaming the cloth bags and different loads and time of pressing (1kg or 2 kg/kg curd for 6 hr and 2 kg/1kg curd for 4hr) to improve the hygienic quality of the end product as compared to conventionally prepared labneh. **Tamime and Crawford (1984)** improved whey drainage using a pressing equipment (@ 0.7-1.1kg/cm^2) in a refrigerated room. Thereafter, the cheese-curd was homogenized resulting in a smoother and finer product.

A method was suggested to produce a yoghurt spread resembling cream cheese by centrifuging a mixture of yoghurt and brine solution to remove bulk whey and obtain soft cream cheese consistency **(Kharrazi, 1984)**. **Dagher and Ali (1985)** fractionated yoghurt prepared from skim milk on a refrigerated centrifuge for 5 min at 4080, 7970 and 1170 x g respectively. The solid sediment comprised the product labneh and the supernatant whey was discarded.

A special device for yoghurt-cheese making has been described by **Grusin (1994).** The device separates whey from yoghurt using an arrangement of angled wire mesh strainers with in square/rectangular container. Whey from yoghurt seeps through the strainer and is collected in the bottom of container.

The use of ultrafiltration (UF) technique for the manufacture of yoghurt cheese has been attempted and two different systems have been used. In the first, the milk is concentrated by UF to derived TS before heat treatment and fermentation **(Abd-El-Salam and EL-Alam, 1982; Ibrahim *et al.*, 1979)**, while the other system involves concentration of warm yoghurt by UF (at 40-50^{0}C) and using a lactic curd homogenizer markedly improved the quality **(Tamime *et al.*, 1989; 1991).**

2.2.7 Shelf life

The shelf life of traditional labneh is poor mainly due to sanitary problems usually associated with the cloth bags used **(Tamime and Robinson, 1978)**. The shelf life could be prolonged if it is heat treated and packed aseptically. **Dagher and Ali (1985)** used mild heat treatment (70^{0}C/3min) in presence of hydrogen - peroxide (0.179%) and addition of potassium sorbate (0.1%) and stabilizers (starch 1% or CMC 0.4%). Such product was reported to have good and acceptable sensory attributes.

Olive oil in the form of a layer has been used to preserve labneh, or ball of labneh-anbaris and extension in shelf life up to 3 months at 25 ^{0}C and one month at room temperature has been reported **(Hassan *et al.*, 1986).**

2.2.8 Nutritive value

Labneh is considered completely as a natural food derived totally from raw dairy materials with no stabilizers or preservatives and is as easy as yoghurt to digest in the stomach **(Tamime 1978; Rao *et al.*, 1987)**. **Mahdi *et al.*, (1990)** suggested that mixing *Bifidobacterium bifidum* with the yoghurt starter culture would overcome the pronounced acetic acid flavor in labneh and maintain the nutritional quality of the product. Addition of P*ropionibacterium frendenrechii* sub sp. *shermanii* to the normal culture during processing of labneh was found to increase the contents of vitamin B_{12} and folic acid in the final product by about 210 and 28 percent respectively **(Khatab, 1991).**

2.3 Functional foods

A food is said to be 'functional'' if it meets one of the following criteria- 1) It contains a food component (being a nutrient or not) which affects one or a limited number of functions in the body in a targeted way so as to have positive effects **(Bellisle et al., 1998)**; 2) It contains physiological effect beyond the traditional nutritional effect **(Clydesdale, 1997)**. Collectively, a functional food should have a relevant effect on the well-being and health or result in a reduction in disease risk.

The component that makes the food 'functional' can be either an essential macronutrient if it has specific physiological effects (as resistant starch or (n-3) fatty acids), or an essential micronutrient if its intake is over and above daily recommendations. Additionally, it could be a food component even though some of its nutritive value is not listed as 'essential' e.g. some oligosaccharide or it is even of non-nutritive value e.g. live microorganisms or plant chemicals. The major role players in development of functional foods are prebiotics, probiotics and synbiotics **(Roberfroid, 1999).**

2.3.1 Production of functional foods

A food is made functional by-

- Increasing the concentration of a natural component, which is effective in inducing the expected effect or, by increasing the concentration of a non-nutritive component for which data demonstrates beneficial effects are available.
- Adding a component that is not normally present in most foods, but for which beneficial effects have been demonstrated e.g. prebiotic fructans
- Replacing a component, usually a macronutrient whose intake is usually excessive and thus has deleterious effects (as fats) with a component for which beneficial effects have been demonstrated e.g. chicory inulin
- Improving the bioavailability of food component that has beneficial effects **(Roberfroid, 1999).**

2.3.2 Target of the effects of functional foods

Targets are colonic micro-flora, the gastro-intestinal physiology, the immune functions, the bioavailability of minerals, the metabolism of lipids and colonic carcinogens **(Roberfroid, 1999).**

2.3.3 Potential health benefits

The major potential health benefits include reduction of risk of colonic diseases, non-insulin dependant diabetes, obesity, osteoporosis and cancer. Scientific research based evidence is required for documentation of health claims and benefits. In some claims, evidences exists while in others, research is still on. Sound hypotheses do already exist for designing the relevant human nutrition trails **(Roberfroid, 1989, 1999).**

2.3.4 Probiotics

Probiotics has been dealt in section 2.1.8

2.3.5 Prebiotics

2.3.5.1 Definition and concept

A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in colon, that can improve the host health **(Gibson and Roberfroid, 1995).**

The prebiotic approach advocates the administration of non-viable entities and therefore overcomes survival problems in upper gastro-intestinal tract. The concept of prebiotics developed as the viability of live bacteria in food products and transit through the gastro-intestinal tract may be variable. Certain oligosaccharides that cannot be digested except through bacterial activity are prebiotics **(Gibson, 1999)**.

2.3.5.2 Criteria for prebiotics

A food ingredient can be deemed as prebiotic if it meets following criteria-

- It must be neither hydrolyzed nor absorbed in the upper part of GI tract.
- It must alter the composition of the colonic micro-biota towards a healthier composition.
- It must be selectively fermented by one or limited number of potentially beneficial bacteria in the colon.
- It must preferably induce effects that are beneficial to the host health.

Any food that reaches the colon such as non-digestible carbohydrates, some peptides and proteins, as well as certain lipids, is a prebiotic candidate. Non-digestible carbohydrates, in particular fructose oligosaccharides, are authentic prebiotics **(Gibson, 1999)**.

2.3.6 Synbiotics

When prebiotics are used in combination with probiotics or live bacteria, the resultant has synergistic effects, referred to as 'synbiotics'. This is because in addition to the action of probiotics that promote the growth of existing strains of beneficial bacteria in the colon, prebiotics such as inulin and oligofructose also act to improve the survival, implantation and growth of newly added probiotics strains **(Niness, 1999).**

Research exists that links the consumption of probiotics and prebiotics decrease risk of colon cancer. The major conclusion from the animal data is that there appears to be synergistic effect of consumption of probiotic bacteria and prebiotic such as fructo-oligosaccharides on the attenuation of the development of colon cancer **(Brady *et al.*, 2000).**

Many European dairy drink and yoghurt manufacturers are using the synbiotic concept. Some of the branded products are 'Probioplus' (Migros, Switzerland), 'Symbalance' (Tonilait, Switerland) and 'Fyos' (Nutica, Belgium) **(Coussement, 1996).**

2.3.7 Inulin and oligofructose

Inulin and oligofructose have attracted much attention recently as non-absorbable carbohydrates with prebiotic properties.

2.3.7.1 Occurrence

Inulin and oligofructose are natural food ingredients commonly found in varying amounts in dietary foods. They are present in more than 36,000 plant species; commonly present in wheat, onion, bananas, garlic, and chicory.

Most of inulin and oligofructose commercially available on the industrial food ingredient market is either synthesized from sucrose or extracted from chicory roots. The roots of chicory, *Cichorium intybus* plant contains ~ 15-30 percent inulin and 5-10 percent oligofructose **(Brady *et al.*, 2000)**.

2.3.7.2 Manufacture of Inulin

The roots of chicory are harvested, sliced and washed. Inulin is extracted from the roots by causing a hot water diffusion process, then purified and dried. It has an average degree of polymerization (DP) of 10-12 and is composed of 2-D-fructofuranoses attached by 2 (1W2) linkages. The chain length varies from 2-60 units. The finished inulin powder typically contains 6-10 percent sugars (glucose, fructose and sucrose). A high performance (HP) type of inulin has also been manufactured by removing the shorter chain molecules. HP-inulin has an average DP of 25 and molecular distribution varying from 11-60. The product is twice more fat mimetic than standard inulin with no sweetness **(Brady *et al.*, 2000)**.

2.3.7.3 Functional properties of inulin

Inulin has high solubility, high thermal stability, gets easily dispersed in liquids, slightly sweet taste, stabilizes foams; and more importantly, it acts as a fat replacer. Inulin has the ability to form micro crystals when dispersed in water or milk. These interact to form smooth creamy texture and provide a fat-like mouth feel. It has been used successfully to replace fat **(Niness, 1999).**

2.3.7.3.1 Fat replacers

Fat in foods contribute to key sensory and physiological benefits. It contributes to flavor or the combined perception of mouth feel, taste and aroma/odor. It also contributes to creaminess, appearance, palatability, texture, and lubricity and increases satiety during meals. It can carry lipolytic flavors. Fat is the most concentrated source of energy in the diet providing 9 Kcal/g **(Ney, 1988; Leland 1997).** High fat intake is associated with increased risk for obesity and some types of cancer, high blood cholesterol and coronary heart disease **(USDA and USDHS, 1995).**

Since the recent past, the acceptance of low fat foods is catching up fast due to increased awareness of their nutritional and health benefits **(Sharma and Kumar, 1996).** Thus foods formulated with fat replacers are an enjoyable alternative to similar high fat foods. By choosing these, health conscious consumers are able to maintain basic foods selection pattern and more easily adhere to low fat diet **(CCC, 1998).**

Fat replacers chemically represent fats, proteins or carbohydrates and are generally categorized in to two groups **(Akoh, 1998)-**

- Fat substitutes- the macromolecules that physically and chemically resemble tri-glycerides, are often referred to as lipid or fat based fat replacers. These are either chemically synthesized or derived from conventional fats and oils by enzymatic modification

- Fat mimetics- the substances that imitate organoleptic or physical properties of tri-glycerides but cannot replace fat on a one- to one basis. These are often called as protein or carbohydrate based fat replacers (e.g.-starch, cellulose). The calorific value thus ranges from 0-4 kcal/g.

2.3.7.4 Inulin as the fat replacer

Inulin is a carbohydrate based fat mimetic, it provides roughly 1kcal/g. It is marketed under the trade name- Raftiline and is used in baked goods, beverage, desserts, fish, meat, poultry products, pasta, processed cheese, yoghurt and a variety of other products **(Sharma and Kumar, 1996)**. It is also used as sugar substitute in chocolate products, for dietary fiber enrichment in milk, yoghurt etc.; dietetic or fibre enriched fruit preparations, reduced fat dressing. In dairy applications, raftiline is used @1-8 percent **(Orafti Manual, 2000).**

It has been often regarded as invisible fiber or prebiotic fiber to replace fat in dairy foods as cheese spreads **(Wouters, 1998).**

2.3.7.5 Manufacture of oligofructose

Oligofructose is derived from chicory in same manner as inulin. The major difference is the addition of hydrolysis step after extraction. Inulin is broken down (partial hydrolysis) using inulase in to chain lengths ranging from 2-10, with an average DP of 4. The resulting oligofructose has ~30 percent sweetness of sucrose and contains ~5 percent glucose, fructose and sucrose on a dry matter basis.

2.3.7.6Functional properties

Like inulin, oligofructose also has high solubility and thermal stability. Furthermore, it is acid stable also. It possesses functional attributes similar to sugar or glucose syrup. Oligofructose contributes body to dairy products, humectancy to soft baked goods, depresses the freezing point in frozen deserts, provides crispness to low fat cookies and acts as a binder in nutritional bars, in much same way as sugar, but with the added benefits of fewer calories, fiber enrichment and other nutritional properties **(Wiedmann and Jager, 1997).**

2.3.7.6.1 Sugar replacers

Sweeteners are the agents that provide sweetness in the product.

These are of two types-

- Bulk sweeteners, which provide calories **(Grenby, 1991) .**

Alternative sweeteners, which have a sweet taste but are essentially non-caloric **(Giese, 1993)**. The latter are in demand to control calorie-intake, prevent tooth decay and facilitate the formation of food products for diabetics **(Grenby, 1991)**. Acesulfame-K, aspartame, saccharine etc are examples of alternative sweeteners.

2.3.7.7 Oligofructose as sugar replacer

Oligofructose is often used in combination with high intensity alternative sweeteners to replace sugar, provide a well-balanced sweetness profile. It also masks the aftertaste of aspartame and acesulfame-K **(Weidmann and Jager, 1997)**. It is used in low-calorie fruit preparations, ice-cream, dairy desserts, functional dairy drinks etc. **(Orafti Manual, 2000)**.

2.3.7.8Therapeutic and nutritional properties of inulin and oligofructose

Therapeutic properties: Inulin and oligofructose are rapidly and completely fermented by the colonic micro flora, with the production of acetate and other short-chain fatty acids **(Jenkins *et al.*, 1999)**. These cause significant increase in colonic bifidobacterial populations thus positively influence the host health **(Gibson and Wang, 1994a; 1994b)**, cause increase delivery of viable bacteria in cultured milk products. Experiments have shown that both stimulate the growth of Bifidobacteria and depress bacteriodes, C*lostrodia* and F*usobacterium*. Both have a favorable influence on lipid metabolism with a decrease of serum tri-glycerides and increase in HDL: LDL ratio, cause a significant increase in retention of calcium, magnesium and iron in rats. These

may also be involved in inhibition of colonic carcinogenesis, blood cholesterol reduction, and immune stimulation and enhanced vitamin synthesis. More research is required to validate these claims. Both are expected to have a disease preventive and treatment implications **(Jenkins *et al.*, 1999)**.

Nutritional properties: Inulin and oligofructose have been used in many countries to replace fat or sugar and reduce the calories of dairy products, confections and baked goods. Inulin and oligofructose have low calorific values than typical carbohydrates due to 2 (2---1) bonds linking the fructose molecules. These bonds render them non-digestible by human intestinal enzymes. Thus, these pass the small intestine unmetabolized. This has been proved by many scientific studies **(Kuppers-Sonnenberg, 1952; Rummessen *et al.*, 1990)**. Studies indicate that almost all inulin or oligofructose ingested enters the colon where it is fermented by colonic microflora. The energy derived from fermentation is largely a result of the production of short-chain fatty acids and lactate, which are metabolized and contribute 1.5 kcal/g of useful energy for both inulin and oligofructose. Non-digestibility makes them suitable for diabetics **(Lewis, 1912; Niness, 1999)**.

Inulin and oligofructose act as dietary fibers, thus influence intestinal function by increasing stool weight, particularly in constipated patients **(Hidaka *et al.*, 1986; Roberfroid, 1999; Niness, 1999). Nilsson *et al.* (1988)** and **Roberfroid (1993)** reported that inulin and oligofructose should be classified as fibers from analytical and physiological point of view.

Perhaps the best known effects of inulin and oligofructose are their actions to stimulate beneficial Bifidobacteria growth in intestine. This action outcomes potential detrimental organisms and thereby potentially contribute to the health of the host **(Roberfroid 1999; Hidaka *et al.*, 1986).**

2.3.8 Protein Isolates and Concentrates

They have the potential to be used in various synbiotic food formulations. Some of these are Whey Protein Isolates (WPI) and Concentrates (WPC) and Soy Protein Isolate (SPI). SPI is being used in majority of research studies on health effects of soy proteins on humans **(Protein Technologies International Manual, 2000).**

2.3.8 Honey

Honey has been used as a potential ingredient in yoghurt formulations in countries like Turkey and New Zealand (Pinar Group of Companies and Biofarm Group of Companies respectively **(Mothey, 2000).**

2.4 Consumer acceptance and physico-chemical and sensory characteristics of low calorie yoghurt and related products

The manufacturers have developed low fat and non-fat yoghurt products with improved compositional and nutritive properties, but many variables affect consumer acceptability of such products. Chemical changes during storage included depletion of lactose, formation of lactic acid, decrease in acetaldehyde and small, but potentially important changes in concentration of

other volatiles **(Laye *et al.*, 1993). Rankin and Brewer, (1998)** reported that fermentation resulted in advantageous changes making non-fat fluid milk color similar to milk fat containing samples.

Low/no fat yoghurts frequently possess a weak body due to lesser of milk solids. Stabilizers are added to provide a firmer gel. Yoghurts made with skim milk fortified with high protein powder and containing up to 5.6 percent protein, 10 percent total solids and 3.75 percent lactose after culturing were found similar in firmness to control yoghurt with 5.1 percent protein, 12 percent total solids and 6 percent lactose. Fat content in both types of yoghurt was less than 0.2 percent **(Mistry and Hassan, 1992).**

The addition of whey protein concentrate (WPC) to milk caused considerable changes in yoghurt components and influenced some taste properties. Addition up to 0.6 percent of WPC improved sensory attributes of yoghurt. Appearance was found better when 1.4-1.6 percent of the same was used **(Penna *et al.*, 1997).**

In an attempt to overcome the problem of whey separation, various stabilizers were added at levels of 0.1, 0.2, 0.3 and 0.4 percent during manufacture of yoghurt from full fat and low fat buffalo milk. Gelatin at 0.2-0.3 percent produced the greatest improvement in yoghurt quality. Sodium-hexa-meta phosphate (0.2-0,3 %), gum acacia (0.2-0.3 %), pectin (0.2%), sodium alginate (0.2%) added individually also improved yoghurt quality,

although gum acacia imparted flavor, thus affecting acceptability of the product **(Shukla and Jain, 1991).**

Addition of fructo-oligosaccharides mixture improved the quality of plain, unsweetened yoghurt. Such product was identified as being brighter in appearance and having a less chalky and creamier texture. It was glossier, sweeter, with a less sour taste and firmer in texture. Fructo-oligosaccharide addition did not result in any significant difference in viable counts and pH; did not degrade shelf life and retained acceptability over expected shelf life of 42 days **(Speigel *et al.*, 1994).**

The viability of *Lactobacillus casei* and *Lactobacillus acidophilus* added as adjuncts were investigated during 28 days of refrigerated storage of yoghurt samples at 5-7 ^{0}C. There was no loss of viability of the adjuncts during storage of yoghurt. *L. acidophilus* MUH-41, 0-16, and L-6 exhibited no significant loss in viability, but strains 4312 and La-5 showed viability loss **(Nighswong *et al.*, 1996).**

Materials & Methods

3. Materials and Methods

3.1 Source of materials

3.1.1 Skim milk

Buffalo milk used in the present investigation was obtained from Verka Milkfed, Milk Plant, Patiala (Punjab), India and Live Stock Research Centre, G.B.Pant University of Agriculture and Technology, Pantnagar, Distt. Udham Singh Nagar (Uttarancal), India.

3.1.2 Cultures

The freeze-dried cultures of *Lactobacillus delbrueckii* sub sp. *bulgaricus-RTS, Streptococcus salivarius* sub sp. *thermophilus-74, Lactobacillus acidophilus-13, Bifidobacterium bifidum*-NCDC-255 were obtained from Culture Collection Centre, National Dairy Research Institute, Karnal (Haryana), India.

The stock cultures of pathogenic organisms grown on nutrient agar slants, namely *Escherichia coli, Staphylococcus aureus and Salmonella havana* were obtained from College of Veterinary Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Distt. Udham Singh Nagar (Uttarancal), India.

3.1.3 Prebiotic and other ingredients

Inulin (Raftiline), the fat replacer and Oligofructose (Raftilose), the sugar replacer, manufactured by M/s ORAFTI-Belgium Active Food Ingredients, Belgium, were obtained from M/s S.A. Chemicals, Goregoan, Mumbai, India. The dietary fibres (oat, orange and apple) were obtained from M/s Clarico-FPC (India) Pvt. Ltd, Mumbai, India. Soy Protein Isolate – SUPRO, honey and Skim milk powder were purchased from M/s Du Pont Protein Technologies International, Gurgoan, Haryana, India; Dabur India Ltd., New Delhi, India and Verka, Milkfed, Milk Plant, Patiala (Punjab), India, respectively.

3.1.4 Chemicals and packaging material

The polyethylene cups were purchased from the local market. The chemicals and media used in the present investigation were of Analytical Reagent (AR) grade.

3.2 Activation of cultures

3.2.1 Activation of starter cultures

The freeze-dried cultures were activated according to the recommendations of suppliers and grown in sterile litmus skim milk at 37 ^{0}C. The cultures were maintained by weekly transfers and stored at 4±1 ^{0}C. These were sub cultured 3-4 times prior to use.

3.2.2 Activation of pathogens

The stock cultures of all pathogenic organisms were grown on nutrient agar slants, transferred at every 7 days and stored at 4±1 ^{0}C between the quarterly transfers. Nutrient broth was used to propagate the organisms in liquid medium.

3.3 Preparation of yoghurt-cheese

Yoghurt-cheese was prepared from the yoghurt prepared by the method described by **Tamime and Robinson (1988)** with modifications with regards to standardization and heat treatment of milk The flow diagram of the method is given in Fig 3.1.

3.3.1.1 Buffalo milk

Fresh buffalo milk having titratable acidity 0.15-0.16 per cent as lactic acid was used throughout the present study.

3.3.1.2 Filtration of the milk

The buffalo milk was filtered through double-layered muslin cloth in order to remove dust, dirt and any other foreign particle.

3.3.1.3 Standardization of the milk

Milk was separated by centrifugal cream separator (Dairy Appliance Limited, Mumbai, India) in order to obtain skim milk. The skim milk thus obtained was used to standardize buffalo milk to 0.5 per cent fat. The

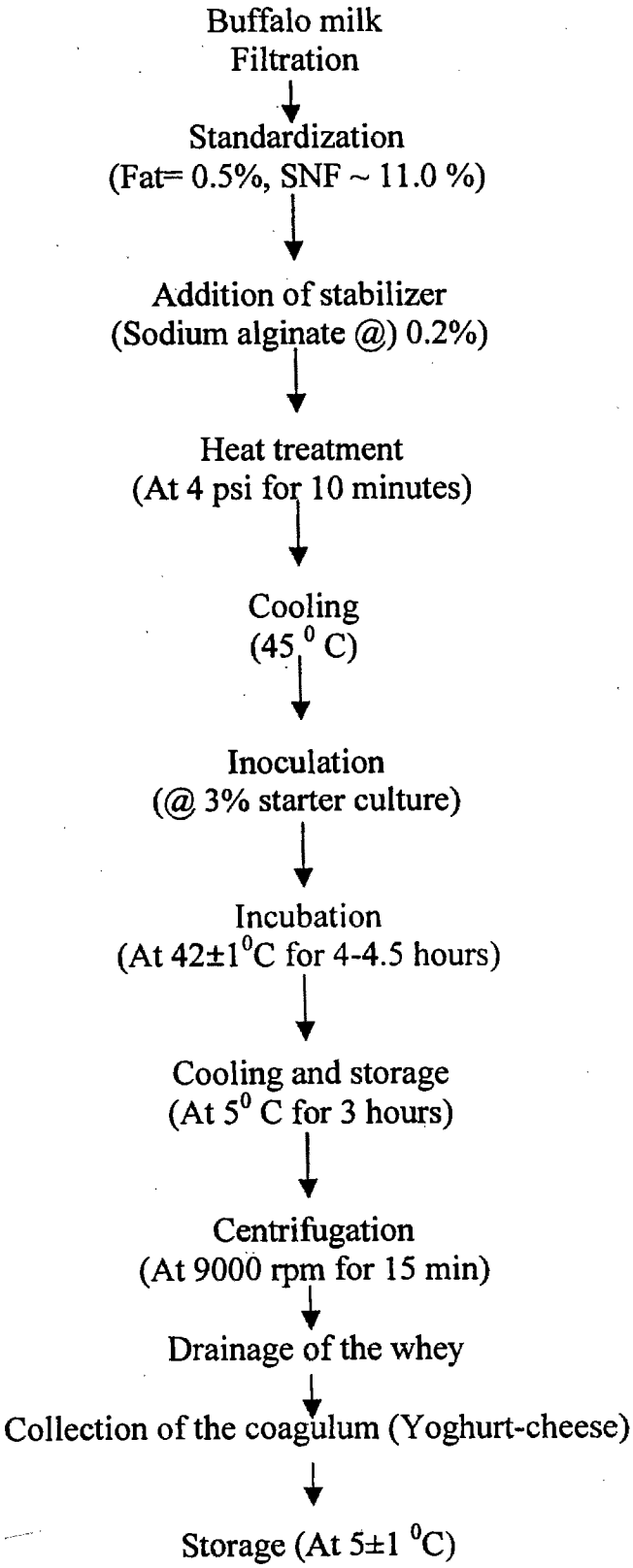

Fig 3.1 General flow diagram for the yoghurt-cheese preparation

standardized milk had approximately 9.0 per cent SNF, the SNF content of the standardized milk was raised to 11.0 per cent by adding 2 per cent skim milk powder (SMP).

3.3.1.4 Heat treatment of the milk

The milk was heated in autoclaveable glass bottles at 4 psi for 10 min in a laboratory autoclave. The heat treatment was optimized by conducting preliminary trials so as to obtain desirable quality yoghurt.

3.3.1.5 Cooling of milk

After heat treatment, the milk was cooled to about 45±1 ^{0}C under running tap water to bring it to the inoculation temperature.

3.3.1.6 Inoculation

Milk was inoculated with 3 per cent bulk yoghurt culture (*Lactobacillus delbrueckii* sub sp. *bulgaricus-RTS* and *Streptococcus salivarius* sub sp *thermophilus-7*). The cultures were grown separately and mixed in the ratio of 1:1 just before inoculation.

3.3.1.7 Incubation

The inoculated milk was incubated at 42±1 ^{0}C for 4-4.5 h or until an acidity of 1.2 to1.3 percent as lactic acid and pH 4.4-4.5 was attained.

3.3.1.8 Cooling and Storage

The yoghurt samples thus prepared were cooled to 5 ^{0}C and stored for about 2 h in order to have a cold induced firm coagulum.

3.3.1.9 Centrifugation

The coagulum was transferred to sterilized centrifugation tubes and centrifuged in a laboratory centrifuge (R24, REMI Instruments, Mumbai, India) at 9000 rpm for 15 min. The speed and time of centrifugation was optimized by conducting preliminary trials so as to obtain maximum cheese yield.

3.3.1.10 Drainage of whey

Upon centrifugation, the curd settled down in the bottom of the centrifuge tubes and a clear whey layer from the top was decanted.

3.3.1.11 Storage of the curd

The cheese curd was transferred to high-density polyethylene cups, covered with aluminum foil and stored under refrigeration at 5±1 ^{0}C till further use.

3.4 Optimization of prebiotic ingredients and enricher level in yoghurt

3.4.1 Optimization prebiotic ingredient level

The following prebiotic ingredients were added to 100 ml of the standardized milk at different levels and mixed thoroughly before heat treatment.

Sl. no.	Prebiotic ingredient	Attempted level (%)
1.	Raftilose	2.5, 3.0, 3.5, 4.0, 4.5
2.	Raftiline	2.5, 3.0, 3.5, 4.0, 4.5
3.	Dietary fibres (apple, oat and orange)	0.5, 1.0, 1.5

The yoghurt was prepared from each milk mix containing different level of prebiotic ingredient in the similar method as described in sections 3.3.1.1. to 3.3.1.8. The fermentation was carried out by yoghurt cultures and the inoculated milk was incubated at 42 ± 1^0 C. The set yoghurt was cooled and then analysed for sensory characteristics on a 9 point Hedonic scale. The level of ingredient in yoghurt, which obtained maximum score for over all acceptability, was considered as optimized level and was thus selected for all further studies.

Addition of apple and orange fibre gave product with unsatisfactory sensory scores. Therefore in all further studies, only oat fibre was used.

3.4.2 Optimization of enricher level

The two enriching ingredients namely soy protein isolate and honey were mixed at different levels to 100 ml of the standardized milk in the following concentration before heating -

Sl. no.	Enriching ingredient	Attempted level (%)
1.	Soy protein isolate	0.5, 1.0, 1.5
2.	Honey	2.0. 2.5, 3.0, 3.5

The yoghurt was prepared in the similar fashion as mentioned in section 3.4.1. The various sets of yoghurt containing different levels of soy protein isolate and honey were subjected to sensory evaluation on 9 point Hedonic scale. The level of enricher in yoghurt, which obtained maximum sensory scores for over all acceptability, was considered as optimized level.

The product prepared by addition of soy protein isolate to milk gave undesirable flavor. However, honey addition improved the flavor.

3.4.3 Optimization of combination of prebiotic ingredients and honey

Attempts were made to combine different prebiotic ingredients with or without honey. Oat fiber was tried to mix with optimized levels of raftiline, raftilose and honey. Thus lower levels of oat fiber (0.25, 0.5 and 0.75%) were selected. Yoghurt samples were prepared by adding optimized levels of raftiline and oat fiber, raftilose and oat fiber, and honey and oat fiber. The ingredients were mixed together and added to 100 ml of standardized milk before heating and the yoghurt was prepared as described in section 3.4.1. The products thus obtained were subjected to sensory analysis in order to obtain the optimal level of two ingredients.

3.5 Preparation of synbiotic yoghurt

Synbiotic yoghurt was prepared by adding optimized levels of prebiotic ingredients and/or enricher to standardized milk before heating and the fermentation was carried out using different set of bacterial cultures having

probiotic bacterial component. The probiotic cultures namely, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were used along with conventional yoghurt cultures for the preparation of synbiotic yoghurt-cheese. The following combinations of cultures were used for the inoculation of standardized milk at the rate of 3 per cent.

- *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius subsp. thermophilus* =1:1 (Control, C_1)
- *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius subsp. thermophilus*: *Lactobacillus acidophilus* = 1:1:1 (C_2)
- *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius subsp. thermophilus*: *Bifidobacterium bifidum* =1:1:1 (C_3)
- *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius subsp. thermophilus*: *Lactobacillus acidophilus: Bifidobacterium bifidum* =1:1:1:1 (C_4)

The synbiotic yoghurt samples so obtained were analysed for physico-chemical characteristics.

3.6 Preparation of synbiotic yoghurt–cheese

Synbiotic yoghurt-cheese samples were prepared after centrifuging the synbiotic yoghurt samples at pre-optimized rpm and the whey was removed from cheese curd. The yoghurt-cheese thus obtained were analysed for physico-chemical characteristics. The recovery of total solids in cheese from

milk was also calculated. The whey obtained during centrifugation was analysed for physico-chemical characteristics.

The biocompatibility studies of bacterial cultures were also carried out.

3.7 Biocompatibility of the bacterial cultures

Biocompatibility studies were carried out for the culture combinations C_1, C_2, C_3 and C_4 as elucidated in section 3.5.

3.7.1 Changes in pH and titratable acidity

The yoghurt-cheese samples were prepared after adding 3 percent of C_1, C_2, C_3 and 4 percent of C_4 culture combinations. The inoculated milk was incubated at 42±1 ^{0} C. The pH and titratable acidity were determined at 0, 4, 8, 12 and 16 hours of incubation. The effect of culture combination on acid production and reduction in pH was studied and data were analysed statistically by 2 factors CRD test.

3.7.2 Bile tolerance

The probiotic cultures were tested for bile tolerance according to the procedure described by **Gilliand and Walker (1990)**. The culture combinations C_1, C_2, C_3 and C_4 were grown in lactic broth **(Ellicker *et al.*, 1956)** with or with out addition of 0.3 and 0.5 per cent bile salt (sodium glycolate, w/v). The inoculated broth tubes were incubated at 37^{0}C for the total period of 16 h. Bacterial growth was determined by means of measuring

milk was also calculated. The whey obtained during centrifugation was analysed for physico-chemical characteristics.

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increase in optical density (OD) at 620 nm after 0, 4, 8, 12 and 16 h of incubation using digital spectrophotometer (Spectrophotometer 106, Systronics, India).

3.7.3 Antagonistic activity of probiotic cultures against different pathogens

The antagonistic activity of probiotic cultures was tested against intestinal and food borne pathogens namely *Escherichia coli, Staphylococcus aureus* and *Salmonella havana.*

3.7.3.1 Preparation of cell free milk culture filtrate

Sterilized skim milk (100 ml) was transferred into each of four 150 ml conical flasks. The flasks were inoculated separately C_1, C_2, C_3 and C_4 @ 2.5 percent. The flasks were then incubated at 37 ^{0}C for 48 h. The curdled milk was then centrifuged at 2000xg for 20 min at 4^{0}C. The supernatant was decanted from the centrifuge tubes and filtered through 0.45-micron filter (Millipore, U.S.A.) in order to obtain cell free filtrate. The filtrate was then tested for inhibitory activity against pathogens by modified agar well assay technique **(BSI, 1968).**

3.7.3.2 Inhibitory activity against pathogens

Seeded plates were prepared by transferring 0.2 ml of active culture (OD 0.45-0.55) of the desired pathogens and then layering with 20 ml of sterilized and cooled nutrient agar (45 ^{0}C). The contents were mixed gently by rotating

the plates. After solidification, the plates were kept at 37 ^{0}C for 2 h and were then punched aseptically using sterile well cutter (10 mm diameter). Thereafter, each well was sealed by pouring some melted agar on the base. A 0.2 ml aliquot of culture filtrate was then poured in each sealed well aseptically. The plates were kept at 4 ^{0}C for 2 h and then incubated at 37 ^{0}C for 24-36 h. At the end of incubation period, the diameter of the zone surrounding the well, if any, was measured using Vernier calipers.

3.8 Storage study

The yoghurt-cheese samples were stored at 5±1 ^{0}C and analysed for physico-chemical, microbiological and sensory characteristics at an interval of 7 days for 4 weeks in order to study life of the product.

3.8.1 Physico-chemical analysis

The milk, yoghurt, yoghurt-cheese and whey samples were analysed for the following physico-chemical attributes.

3.8.1.1 Moisture content

Moisture content of the sample was determined by **AOAC (1984)** procedure. The sample (10 g) was weighed accurately and transferred to a cleaned, dried and weighed dish. The contents were dried in a hot air oven at 100 ^{0}C until a constant weight. After cooling, the loss in weight was taken as moisture content and expressed in terms of percentage.

$$\text{Moisture (\%)} = \frac{\text{Initial weight of the sample} - \text{Weight of the dried sample}}{\text{Initial weight of the sample}} \times 100$$

3.8.1.2 Protein

Protein estimation was done by Micro-kjeldhal method **(AOAC, 1984)**. 2 g of the sample was transferred to a digestion flask followed by addition of 3 g of digestion mixture ($K_2 SO_4$: $CuSO_4$: SeO_2 in 100:20:2 ratio) and 40 ml of concentrated sulphuric acid. The contents were then digested till a blue-green transparent liquid was obtained. The contents were then transferred to a 100 ml volumetric flask and volume was made up to 100 ml using glass-distilled water. A 10 ml of the aliquot of digested mixture was distilled with excess of 40 per cent NaOH and the liberated ammonia was collected in 30 ml of 4 per cent boric acid solution containing 2-3 drops of mixed indicator (10 ml of 0.1 percent bromocresol green + 2 ml of 0.1 per cent methyl red indicator in 95 per cent ethyl alcohol). The entrapped ammonia was titrated against 0.1 N HCl. A blank was simultaneously digested and distilled. Nitrogen content in the sample was calculate as follows-

$$\text{\% Nitrogen} = \frac{14 \times (\text{Sample titre-Blank titre}) \times \text{Normality of HCl} \times \text{Vol.made up of aliquot}}{1000 \times \text{weight of the sample (g)} \times \text{Aliquot of digest taken}} \times 100$$

$$\text{\% Protein} = \text{\% Nitrogen} \times 6.38$$

3.8.1.3 Fat

3.8.1.3.1 Milk and whey sample

The percent fat in milk samples was determined by Gerber method **(SP-1224 (Part I), 1977)** as outlined below.

Ten ml of sulphuric acid (90:10, v/v with water) was taken in the butyrometer using an automatic pipette. The sample was then poured drop-by-drop using 10.75 ml pipette. 1 ml of amyl alcohol was added and the mouth of the butyrometer was closed with the help of stopper and key. The contents were mixed and shaken carefully and then centrifuged in a horizontal centrifuge for 5 min. The percent fat was then noted directly from the butyrometer calibrations.

3.8.1.3.2 Yoghurt and yoghurt-cheese sample

Fat content of the sample was determined by Soxhlet Extraction method **(AOAC, 1984)**. 5 g of the sample was dried at 100 ^{0}C for 3 h and transferred to a thimble. The thimble was placed in the Soxhlet apparatus and fat was extracted for 16 -18 h with petroleum ether (B.P. 60-80 ^{0}C). Thereafter, the ether was evaporated and the extracted fat was weighed. Fat percentage was calculated by the following expression.

$$\% \text{ Fat} = \frac{\text{Weight of the ether extract}}{\text{Weight of the sample}} \times 100$$

3.8.1.3 Ash

Ash content in the samples was determined according to **AOAC (1984)** method. 10 g of sample was weighed in to a silica dish, charred and ignited in muffle furnace at 550^0C until carbon free ash was obtained. The dish was cooled in desiccators and weighed.

$$\text{Ash (\%)} = \frac{\text{Weight of the residue}}{\text{Weight of the sample}} \times 100$$

3.8.1.4 Carbohydrate content

The carbohydrate content was calculated by difference method. The sum of moisture, protein, fat and ash content was subtracted from hundered and the result was designated as percent carbohydrate content.

3.8.1.5 pH

pH of the milk, yoghurt and whey samples was measured directly using the digital pH meter (Electronics Corporation of India Limited, Hyderabad).

For yoghurt-cheese sample, 2 g of the sample was first mixed well with 10 ml of glass-distilled water and then the pH was noted as above.

3.8.1.6 Titratable acidity

Titratable acidity (as % lactic acid) of the samples was determined according to **AOAC (1984)** method. The milk and whey samples (10ml) were

directly titrated against 0.1 N NaOH to pH 8.4 using phenolpthalein as indicator to a faint pink end point.

Yoghurt and yoghurt-cheese samples (10ml and 10 g respectively) were diluted two and ten times, respectively, with glass distilled water and thoroughly mixed before titration.

The results were expressed as percentage lactic acid.

$$\% \text{ TA} = \frac{9 \times \text{titre value} \times \text{Normality of NaOH} \times \text{Dilution factor}}{\text{Weight of the sample taken (gm)}}$$

3.8.1.7 Soluble nitrogen

The soluble nitrogen in the samples was estimated according to **Kosikowski (1977)**. The sample (5 g) was transferred in to volumetric flask followed by a small amount of Sharp's extraction solution (tampered to 40^0C) and the contents were mixed. Later on, more Sharp's extraction solution was added to make a dilute suspension and the volume was made to 100 ml using distilled water. Thereafter, the flask was kept in water bath at 50^0C for 1 h with occasional shaking. The contents were filtered through Whatman filter paper 42. The filtrate (25 ml) was transferred to a 300 ml kjeldhal flask, digested, distilled and titrated to determine nitrogen. The soluble nitrogen was calculated as follows –

$$\% \text{ Nitrogen} = \frac{14 \times (\text{Sample titre-Blank titre}) \times \text{Normality of HCl} \times \text{Vol. made up of aliquot}}{1000 \times \text{Weight of the sample (gm)} \times \text{Aliquot of digest taken}} \times 100$$

The composition of the reagent was as under:

Stock A: 57.5 ml Glacial acetic acid, 47.0 g Sodium Chloride, 136.1 g Sodium Acetate, 8.9 g Calcium Chloride. All the constituents were dissolved in distilled water and the final volume was made up to 1 liter.

Sharp's Extraction Solution: 250 ml of Stock A was diluted to 1 liter in distilled water.

3.8.2 Microbiological

The microbiological analysis was carried out according to the procedure given in A.P.H.A. (1992). Sample (1 g) was weighed aseptically and suspended uniformly in 9 ml of saline dilution blank. Serial dilutions were also prepared. In each count, after incubation, the average count of colonies present on the petriplates were multiplied by dilution factor and expressed as colony forming units (cfu) per gram.

3.8.2.1 Total Viable Count

The viable count in the yoghurt–cheese samples was determined according to **A.P.H.A. (1992)** procedure using lactic agar **(Ellicker *et al.*, 1956)** at an interval of 7 days. Duplicate plates were prepared using appropriate dilutions, incubated at 37^0C for 24-48 h. The colonies (30-300 in number) were counted and the number (organism/g of sample) was determined by multiplying it with dilution factor.

3.8.2.2 Yeast and Mold Count

The yeast and mold count was conducted according to **SP:18, (Part I), ISI, 1980** method. Potato dextrose agar (Hi-Media Laboratories Pvt. Ltd., Mumbai) acidified to pH 3.5 with sterile 10 per cent tartaric acid was used to innumerate the count. Incubation was carried out at 22 ± 1^0 C for 2-4 days.

3.8.2.3 Proteolytic count

One ml of sample of appropriate dilution was plated with skim milk agar prepared by mixing plate count agar (Hi-Media Laboratories Pvt. Ltd., Mumbai) and reconstituted skim milk (10 % total solids) in ratio of 10:1. The petriplates were incubated at 37 ^{0} C for 24-48 h. At the end of incubation, petriplates were flooded with excess of 1 per cent HCl for one minute. Colonies surrounded by clear zone produced by proteolytic organisms were counted.

3.8.2.4 Coliform Count

The coliform count in the yoghurt –cheese samples was determined according to **A.P.H.A. (1992).** Appropriate dilution of 1 gm of the sample was plated with 10-15 ml of violet red bile agar (VRBA) (Hi-Media Laboratories Pvt. Ltd., Mumbai) in duplicate. After solidification, the plates were overlaid with 3-5 ml of additional VRBA. The plates were incubated at 35 ^{0} C for 48 h after solidification. Purplish red colonies of 0.5 mm diameter or larger, surrounded by a reddish zone of precipitate bile were counted. For confirmation, 10 representative colonies from plates were transferred to

separate tubes containing 10 ml of brilliant green bile broth and a Durhum's tubes. The test tubes were incubated at 35^0C for 48 h and examined for evidence of gas production. The number of coliform colony forming units (cfu/gm) of the sample was determined by multiplying the original average count with percentage positive tubes and dilution factor.

3.8.3 Sensory Evaluation

The yoghurt and yoghurt-cheese samples were evaluated for their sensory characteristics namely color, flavor, body and texture and over all acceptability using semi-trained panel comprising of 10 panelists drawn from faculty members and post graduate scholars of School of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab and Department of Food Science and Technology, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttaranchal. The panelists were asked to record their observations on the sensory sheet based on 9 Point Hedonic Scale with the following levels-

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3

Dislike very much	2
Dislike extremely	1

3.8.4 Statistical Analysis

Statistical procedures as described by **Snedecor and Cochran (1977)** were used to analyze data for interpretation of results. Mean, Standard deviation, analysis of variance (ANOVA) and least significant difference among the treatments were determined.

Results & Discussion

4. Results and Discussion

The present investigation was envisaged to optimize the parameters for the preparation of synbiotic yoghurt-cheese from standardized buffalo milk containing 0.5 per cent fat. Various prebiotic ingredients namely raftiline (the fat replacer), raftilose (the sugar replacer), apple, orange and oat fiber and enrichers namely soy-protein-isolate and honey were added to milk singly or in combination. The fermentation was carried out with probiotic cultures to prepare synbiotic yoghurt-cheese. The probiotic cultures namely *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were used along with the conventional yoghurt starters- *Lactobacillus delbrueckii* sub sp. *bulgaricus* and *Streptococcus salivarius* sub sp. *thermophilus* in different combinations. The levels of prebiotic ingredients were optimized on the basis of sensory characteristics of yoghurt. The yoghurt samples prepared from milk containing optimized levels of prebiotic ingredients singly or in combination were analysed for proximate composition. The yoghurt-cheeses prepared by centrifugation of yoghurt samples and the whey samples obtained were also analysed for proximate composition. The bacterial cultures were subjected to biocompatibility studies. The yoghurt-cheeses prepared from milk containing optimized levels of prebiotic ingredients/enrichers and different combinations of bacterial cultures were stored at 5 ± 1^{0}C. The physico-chemical, microbiological and sensory characteristics of the product were determined at

an interval of 7 days to assess the shelf life of the product. The experiments were conducted in triplicate and the results presented below are average of triplicate determinations.

4.1 Composition of standardized buffalo milk

Buffalo skim milk was obtained after separating fat from whole buffalo milk using a centrifugal cream separator. Whole buffalo milk was then standardized to 0.5 per cent fat with buffalo skim milk. The composition of whole and standardized buffalo milk is given in Table 4.1. The standardized milk contained about 9.6 per cent total solids. The protein, fat, lactose and ash content of standardized buffalo milk were 3.49, 0.5, 4.9 and 0.71 per cent, respectively. The values are in consonance with the values reported by **De (1980).**

The SNF content of standardized buffalo milk was raised by addition of 2 per cent skim milk powder in order to obtain a firm gel of yoghurt. Sodium alginate @ 0.2 percent was added as stabilizer.

Table 4.1 Composition of whole and standardized buffalo milk

Constituent	Quantity (%)	
	Whole Buffalo milk	Standardized buffalo milk
Moisture	85.8	90.4
Protein	3.44	3.49
Fat	5.19	0.5
Lactose	4.87	4.9
Minerals (ash)	0.70	0.71
pH	6.7	6.7
TA (%lactic acid)	0.16	0.16

4.2 Optimization of heat treatment of milk

The standardized buffalo milk was heated in an autoclave at 2, 3, 4 and 5 psi for 5 and 10 min and the quality of milk was assessed organoleptically for flavour and browning. The milk was stored under refrigeration at 5 ^{0} C and the shelf life of milk was noted. It was revealed that milk heated at 4 psi (~90^{0}C) for 10 min, had desirable flavour and very little browning. Such milk could be stored under refrigeration for more than 15 days without much effect on its sensory quality. The open pan heating of milk was also attempted, but it resulted more browning and development of high intensity of cooked flavour. The problem of scum formation was also observed in open pan heating of milk.

4.3 Optimization of prebiotic ingredient/enricher level in milk

4.3.1 Raftiline

Raftiline, the fat replacer, was added to standardized milk at the levels of 0.0, 2.5, 3.0, 3.5, 4.0 and 4.5 per cent (w/v) and the yoghurt prepared was evaluated organoleptically for colour, flavour, texture and overall acceptability (Table 4.2). The statistical analysis of results revealed that addition of raftiline to standardized milk before yoghurt preparation did not alter its colour significantly. However, the sensory scores of flavour, texture and overall acceptability improved significantly ($P \leq 0.01$) by increasing the level of raftiline up to 4 per cent. But further increase in level of raftiline in standardized milk gave yoghurt with decreased sensory quality with respect to flavour, texture and overall acceptability.

Amiri (2001) obtained the maximum sensory score for overall acceptability of yoghurt prepared after adding 3.25 per cent raftiline in milk. She optimized the level of raftiline in milk for yoghurt preparation by application of Response Surface Methodology.

4.3.2 Raftilose

The addition of raftilose, the sugar replacer, to milk before yoghurt preparation also did not alter sensory scores of yoghurt for colour significantly (Table 4.3). However, the sensory scores for flavour, texture and overall acceptability of yoghurt were increased significantly ($P \leq 0.01$) by increasing the level of raftilose in milk up to 4.0 per cent (w/v). The further increase in its level, however, adversely affected the sensory quality of yoghurt with respect to flavour, texture and overall acceptability (Table 4.2.2). Therefore, a 4 per cent level of raftilose was selected for all further studies.

Amiri (2001) however optimized a level of 3.25 per cent raftilose in milk for yoghurt preparation by application of Response Surface Methodology.

4.3.3 Dietary fibers

Three sources of fiber namely oat, apple and orange were tried for addition in milk for yoghurt preparation, but only oat fiber gave an acceptable product. Oat fiber was added to standardized milk to the extent of 0.0, 0.5, 0.75 and 1.0 per cent (w/v) for yoghurt-preparation and the product was evaluated for colour, flavour, texture and overall acceptability. The addition of oat fiber to milk before yoghurt preparation decreased the sensory scores of yoghurt for

Table 4.2 Effect of raftiline level in milk on the sensory quality of yoghurt

Raftiline added (%)	Sensory Scores			
	Colour	Flavour	Texture	OAA
0.0 (control)	8.12	7.07	6.90	6.95
2.5	8.13	7.02	7.15	6.74
3	8.12	7.38	7.24	7.05
3.5	8.17	8.07	7.61	7.22
4	8.33	8.51	8.47	8.50
4.5	8.13	7.68	8.24	8.01
Grand Mean	8.12	7.61	7.60	7.41
F value	ns	**	**	**
SEM±	0.034	0.026	0.029	0.026
Cd (P≤0.01)	-	0.099	0.111	0.100

Scores expressed on a 9 Point hedonic scale
ns : Non-significant
**: Highly significant
OAA: Overall acceptability

Table 4.3 Effect of raftilose level in milk on the sensory quality of yoghurt

Raftilose added (%)	Sensory Scores			
	Colour	Flavour	Texture	OAA
0.0 (control)	8.12	7.07	6.90	6.95
2.5	8.12	7.01	6.71	6.74
3	8.08	7.49	7.00	7.25
3.5	8.12	7.72	7.51	7.73
4	8.15	8.45	8.42	8.26
4.5	8.11	7.76	8.01	8.01
Grand Mean	8.13	7.58	7.42	7.79
F value	ns	**	**	**
SEM±	0.031	0.025	0.034	0.022
Cd (P≤0.01)	-	0.095	0.119	0.083

Scores expressed on a 9 Point hedonic scale
ns : Non-significant
**: Highly significant
OAA: Over allacceptability

colour significantly ($P \leq 0.01$) (Table 4.4). However, the sensory scores for flavour, texture and over all acceptability increased significantly ($P \leq 0.01$) upto 1.0 per cent addition of oat fiber to milk. Further increase in oat fiber levels in milk caused a significant ($P \leq 0.01$) decrease in the sensory scores of flavour, texture and overall acceptability.

4.3.4 Soy protein isolate

Attempts were also made to enrich the yoghurt by adding soy protein isolate (SPI) in order to improve its nutritional quality. However, addition of SPI to milk for yoghurt preparation adversely affected all the sensory characters namely colour, flavour, texture and overall acceptability. The sensory scores of SPI added yoghurt were much lower in comparison to control yoghurt (Table 4.5). Thus it was not used further in the investigations.

4.3.5. Honey

Honey was added to milk as an enricher in order to improve flavour and texture of yoghurt. It was added to standardized milk at the level of 0.0, 2.0, 2.5, 3.0 and 3.5 per cent (v/v) before yoghurt preparation. Yoghurt obtained was evaluated for sensory attributes namely colour, flavour, texture and over all acceptability (Table 4.6). The sensory scores for colour decreased whereas sensory scores for flavour, texture and overall acceptability increased significantly ($P \leq 0.01$) with increasing concentration of honey up to 3 per cent. However, addition of 3.5 per cent honey to standardized milk before yoghurt

Table 4.4 Effect of oat fibre level in milk on the sensory quality of yoghurt

Oat fiber added (%)	Sensory scores			
	Colour	Flavour	Texture	OAA
0.0 (control)	8.12	7.07	6.90	6.95
0.5	7.67	7.25	7.00	7.00
1.0	7.50	7.50	7.25	7.26
1.5	7.49	6.72	6.50	6.49
Grand Mean	7.70	7.13	6.91	6.93
F value	**	**	**	**
SEM±	0.036	0.023	0.029	0.026
Cd (P≤0.01)	0.142	0.092	0.114	0.103

Scores expressed on a 9 Point hedonic scale
ns : Non-significant
**: Highly significant
OAA: Overall acceptability

Table 4.5 Effect of soy protein isolate level in milk on the sensory quality of yoghurt

SPI added(%)	Sensory scores			
	Colour	Flavour	Texture	OAA
0.0 (control)	8.12	7.07	6.90	6.95
0.25	7.58	6.71	7.05	6.80
0.5	6.97	6.48	7.05	6.57
1.0	6.91	5.90	6.64	6.14
Grand Mean	6.54	6.53	6.90	6.62
F value	**	**	**	**
SEM±	0.028	0.028	0.037	0.034
Cd (P≤0.01)	0.109	0.109	0.146	0.132

Scores expressed on a 9 Point hedonic scale
ns : Non-significant
**: Highly significant
OAA: Overall acceptability

Table 4.6 Effect of honey level in milk on the sensory quality of yoghurt

Honey added (%)	Sensory scores			
	Colour	Flavour	Texture	OAA
0.0 (control)	8.12	7.07	6.90	6.95
2.0	7.73	7.73	7.00	7.00
2.5	7.73	7.98	6.90	7.25
3.0	7.96	8.24	7.12	7.99
3.5	7.83	7.87	7.70	7.44
Grand Mean	7.88	7.78	6.98	7.73
F value	**	**	ns	**
SEM±	0.029	0.026	0.028	0.030
Cd (P≤0.01)	0.112	0.102	-	0.117

Scores expressed on a 9 Point hedonic scale
ns : Non-significant
**: Highly significant
OAA: Overall acceptability

Table 4.7 Effect of raftiline and oat fiber level in milk on the sensory quality of yoghurt

Raftiline + Oat fiber added (%)	Sensory scores			
	Colour	Flavour	Texture	OAA
0.0 (control)	8.12	7.07	6.90	6.95
(4+0.25)	7.93	7.84	8.13	8.09
(4+0.5)	7.77	8.15	8.26	8.25
(4+0.75)	7.63	7.87	8.13	7.98
Grand Mean	7.87	7.73	7.86	7.83
F value	**	**	**	**
SEM±	0.049	0.039	0.039	0.035
Cd (P≤0.01)	0.109	0.151	0.153	0.137

Scores expressed on a 9 Point hedonic scale
ns : Non-significant
**: Highly significant
OAA: Overall acceptability

preparation resulted in slightly lower sensory score of flavour and over all acceptability due to the presence of detectable honey flavour, which masked the original flavour of yoghurt.

4.3.6 Combination of prebiotic ingredients and/or enricher

Attempts were made to use two ingredients simultaneously in order to incorporate nutritional and functional qualities of both ingredients in the yoghurt and subsequently in yoghurt-cheese.

Oat fiber was added along with optimized level of raftiline, raftilose and honey. However, the levels of oat fiber in combination used were 0.25, 0.5 and 0.75 per cent (w/v). Addition of oat fiber along with 4 per cent raftiline (Table 4.7) and 4 per cent raftilose (Table 4.8) and 3 per cent honey (Table 4.9) resulted in yoghurt with significantly ($P \leq 0.01$) inferior colour. However, the flavour, texture and overall acceptability of yoghurt improved significantly ($P \leq 0.01$) upto 0.5 per cent level of oat fiber and decreased thereafter. The reduction in flavour and over all acceptability was observed probably due to the presence of flavour of additive, which masked the original flavour of yoghurt.

4.3.7 Effect of prebiotic ingredients or enricher addition on sensory score of yoghurt

4.3.7.1 Colour

The effect of prebiotic ingredients or enricher addition on sensory scores of yoghurt for colour is given in Fig 4.1. No significant effect of addition of

Table 4.8 Effect of raftilose and oat fiber level in milk on the sensory quality of yoghurt

Raftilose + Oat fiber added (%)	Sensory scores			
	Colour	Flavour	Texture	OAA
0.0 (control)	8.12	7.07	6.90	6.95
(4+0.25)	8.07	7.90	8.09	8.16
(4+0.5)	7.87	8.05	8.16	8.15
(4+0.75)	7.61	7.83	7.94	7.89
Grand Mean	7.93	7.71	7.77	7.81
F value	**	**	**	**
SEM±	0.027	0.045	0.040	0.034
Cd(P≤0.01)	1.045	0.174	0.158	0.132

Scores expressed on a 9 Point hedonic scale
ns : Non-significant
**: Highly significant
OAA: Overall acceptability

Table 4.9 Effect of honey and oat fiber level in milk on the sensory quality of yoghurt

Honey + Oat fiber added (%)	Sensory scores			
	Colour	Flavour	Texture	OAA
Control	8.121	7.072	6.900	6.95
(3+0.25)	8.072	7.950	7.892	8.04
(3+0.5)	7.905	8.050	8.077	8.11
(3+0.75)	7.638	7.783	7.911	7.83
Grand Mean	7.944	7.713	7.695	7.74
F value	**	**	**	**
SEM±	0.032	0.041	0.043	0.039
Cd (P≤0.01)	0.126	0.162	0.168	0.153

Scores expressed on a 9 Point hedonic scale
ns : Non-significant
**: Highly significant
OAA: Overall acceptability

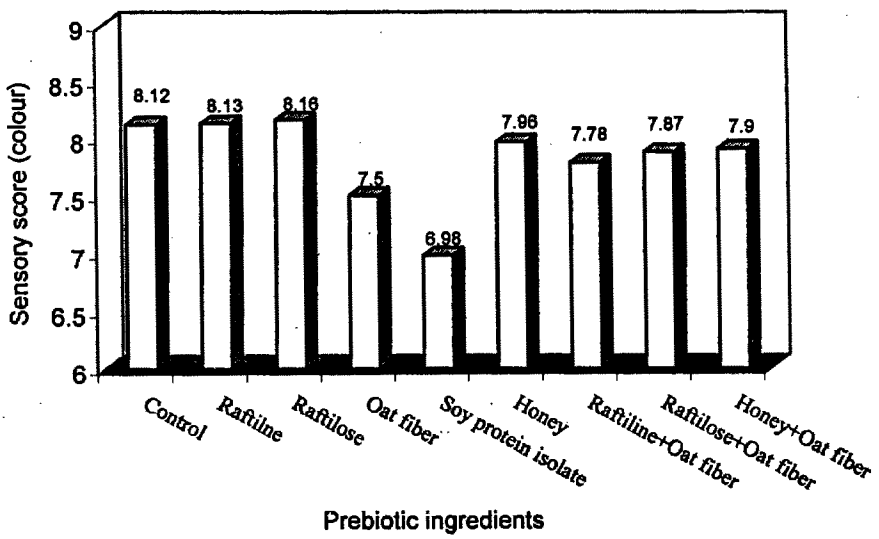

Figure 4.1 Mean sensory scores for colour of yoghurt prepared from optimized level of prebiotic ingredients

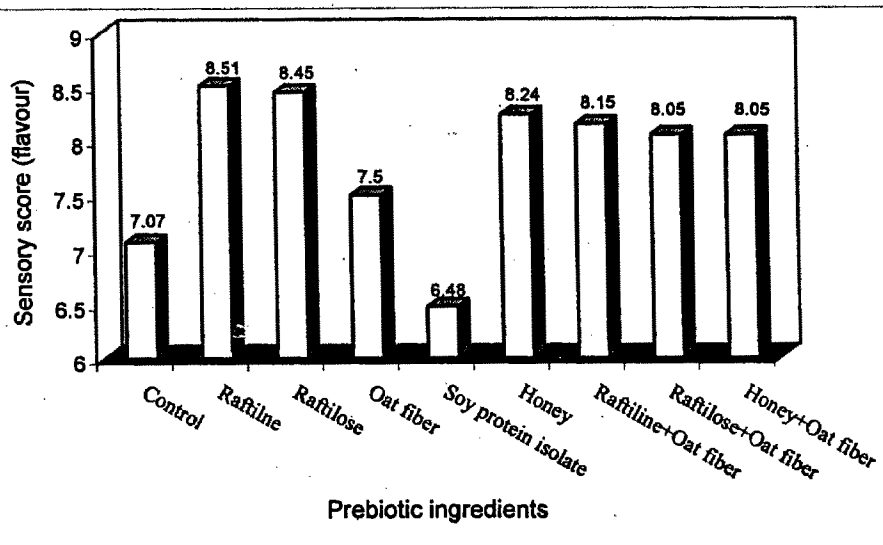

Figure 4.2 Mean sensory scores for flavour of yoghurt prepared from optimized level of prebiotic ingredients

raftiline and raftilose was observed for sensory scores for colour of yoghurt. However, the sensory scores for colour of yoghurt decreased significantly by addition of oat fiber, soy protein isolate, honey and combinations of oat with raftiline, raftilose and honey. The minimum score for colour was obtained in case of soy protein isolate followed by oat and honey.

4.3.7.2 Flavour

The effect of prebiotic ingredients or honey addition on the sensory scores for flavour of yoghurt is shown in Fig 4.2. The sensory scores for flavour increased significantly ($P \leq 0.01$) by addition of all the prebiotic ingredients. However, addition of soy protein isolate significantly ($P \leq 0.01$) reduced sensory score for flavour of yoghurt. The highest score was obtained for yoghurt when raftiline was added followed by raftilose and honey. Among the prebiotic additives, the minimum improvement in flavour of yoghurt was obtained by the addition of oat fiber. The combinations of prebiotic additives express the combined effect of both additives on flavour of yoghurt.

4.3.7.3 Texture

The texture of yoghurt also improved significantly by adding prebiotic additives and honey (Fig. 4.3). Among all the additives, the highest increase in texture of yoghurt was observed in case of raftiline, followed by raftilose, oat, honey and soy protein isolate. On combining raftiline and raftilose with oat fiber, slight reduction in sensory score for yoghurt was obtained. But when

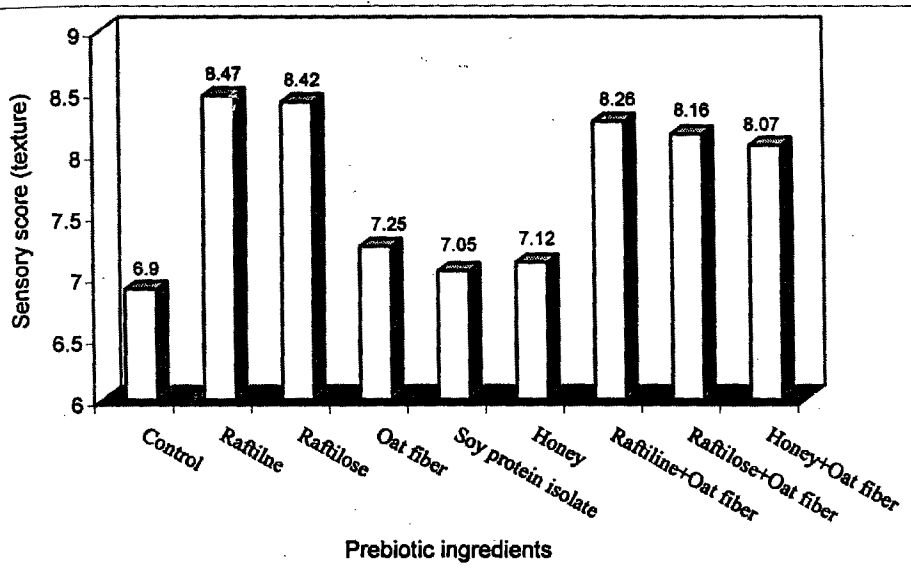

Figure 4.3 Mean sensory scores for texture of yoghurt prepared from optimized level of prebiotic ingredients

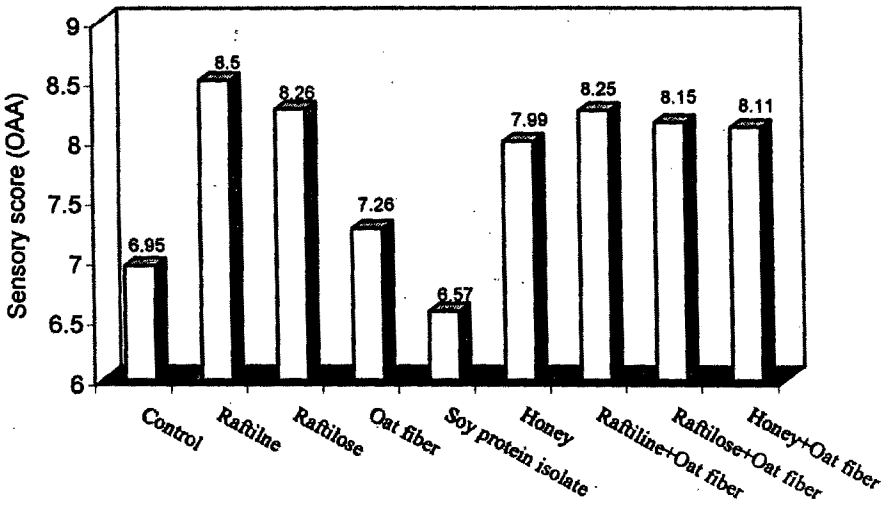

Figure 4.4 Sensory scores for over all acceptability of yoghurt prepared from optimized level of prebiotic ingredients

honey and oat fiber were added together, the sensory scores for texture improved over the individual addition of additive.

4.3.7.4 Overall acceptability

The sensory scores for overall acceptability of yoghurt also increased significantly ($P \leq 0.01$) by addition of prebiotic ingredients or enricher singly or in combination, except in case of soy protein isolate which reduced the sensory score for over all acceptability of yoghurt significantly ($P \leq 0.01$) due to its peculiar flavour (Fig. 4.4). The highest increase in sensory score of over all acceptability of yoghurt was obtained in case of raftiline followed by raftilose, honey and oat fiber. Combination of oat fiber with other ingredients also showed the same trend.

4.4 Synbiotic yoghurt

4.4.1 Effect of bacterial culture and prebiotic ingredients or enricher addition on physico-chemical composition of synbiotic yoghurt

Optimized levels of prebiotic ingredients/enricher were added to the standardized milk and the fermentation was carried out with four sets of starter cultures. The products obtained were analysed for physico-chemical characteristics and the results obtained are presented in Table 4.10-4.16.

4.4.1.1 Moisture content

As shown in Table 4.10, the moisture content of the yoghurt ranged between 86.58 to 88.98 per cent. It was observed that only the additives

Table 4.10 Effect of bacterial culture and prebiotic ingredients/enricher on the moisture content of yoghurt

Bacterial Culture$(M)	% Moisture in yoghurt							
	Prebiotic ingredients/enricher added (S)							
	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4%+ .5%)	Raftilose + Oat fibre (4% + .5%)	Honey + Oat fibre (3% +. 5%)
C_1	88.96	86.91	86.92	86.58	87.81	86.88	86.99	87.16
C_2	88.98	86.88	86.88	86.69	87.71	86.88	86.86	87.10
C_3	88.98	86.66	86.88	86.76	87.81	86.81	86.85	87 .04
C_4	88.91	86.88	86.87	86.69	87.83	86.84	86.84	87.00
Grand Mean	88.96	87.02	86.89	86.68	87.79	86.85	86.88	87.07

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.029	-
S	**	0.044	0.169
M x S	ns	0.088	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Incubation temperature: 42±1 ^{0}C

Enricher

significantly ($P \leq 0.01$) decreased the moisture content of yoghurt. The type of culture and the interaction between the type of culture and prebiotic ingredients/enricher did not affect the moisture content of yoghurt. Among the additives, raftiline, raftilose and oat fiber caused a greater decrease in moisture content of yoghurt when compared with enricher honey. Similar trend was observed when oat fiber was added along with raftiline, raftilose and honey (Fig. 4.5). Such result might have been observed probably due to more compact gel formation after addition of the prebiotic ingredients and honey, which also increased the total solid content of resultant yoghurt.

Tamime *et al.* (1987) have reported a range of 81.3 to 88.0 per cent moisture (12-18.7% total solids) from the result of a survey for natural set/stirred yoghurt. **Tamime and Deeth (1980)** reviewed the standards for chemical composition of yoghurt in various countries and have reported a range of 84 to 91.75 per cent moisture (8.25-16% total solids). **Amiri (2001)** reported 90 per cent moisture in control yoghurt prepared from skim milk without addition of skim milk powder.

4.4.1.2 Protein content

The protein content of yoghurt was found to depend upon its moisture content and the type and amount of prebiotic ingredient or enricher added to the standardized milk. However, differences in the protein content of yoghurts were not statistically significant (Table 4.11). The protein content of yoghurt was between 4.30 and 4.96 per cent. Fig. 4.6 shows that the protein content

Table 4.11 Effect of bacterial culture and prebiotic ingredients/enricher on the protein content of yoghurt

Bacterial Culture$(M)	% Protein in yoghurt							
	Prebiotic ingredients/enricher added (S)							
-	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4% +.5%)	Raftilose + Oat fibre (4% +.5%)	Honey + Oat fibre (3% +.5%)
C_1	4.31	4.69	4.88	4.78	4.81	4.95	4.79	4.84
C_2	4.30	4.72	4.91	4.81	4.79	4.88	4.88	4.96
C_3	4.30	4.78	4.76	4.75	4.83	4.86	4.85	4.80
C_4	4.31	4.71	4.80	4.72	4.82	4.77	4.82	4.79
Grand Mean	4.31	4.72	4.83	4.76	4.81	4.86	4.83	4.84

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.029	-
S	ns	0.041	-
M x S	ns	0.083	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Incubation temperature: 42±1 ^{0}C

Enricher

was minimum in control yoghurt samples as compared to the samples prepared after addition of prebiotic additives and/or honey. These differences may be inversely correlated with moisture content of yoghurt.

Tamime and Robinson (1988) have reported a range of 4.5 to 6.4 per cent protein in natural set/stirred yoghurt. **Amiri (2001)** has reported 3.3 per cent protein in control yoghurt. Such a difference in protein content of yoghurt might have been observed in the present investigation due to variations in composition of milk used and amount of skim milk powder added to milk.

4.4.1.3 Fat content

As shown in Table 4.12, no significant difference was observed in the fat content of yoghurt samples prepared after adding different prebiotic ingredients and honey. The type of bacterial culture and their interaction with ingredients also had a non-significant effect on the fat content of yoghurt. However, the fat content in yoghurt was slightly higher as compared to milk used for its preparation. This increase may be attributed to the evaporation of moisture from milk during heating and incubation at higher temperature (42±1°C). To the standardized milk containing 0.5 per cent fat, 2 per cent skim milk powder was also added before yoghurt preparation in the present investigation, which contained about 1.0 per cent fat. Fig. 4.7 also shows slight variations in fat content of yoghurt prepared from milk containing different prebiotic ingredient and honey. However, these differences were statistically non-significant.

Table 4.12 Effect of bacterial culture and prebiotic ingredients/enricher on the fat content of yoghurt

Bacterial Culture$(M)	% Fat in yoghurt							
	Prebiotic ingredients/enricher added (S)							
	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4% +.5%)	Raftilose + Oat fibre (4% +.5%)	Honey + Oat fibre (3% +.5%)
C_1	0.81	0.80	0.80	0.80	0.80	0.81	0.82	0.81
C_2	0.80	0.81	0.80	0.80	0.80	0.79	0.80	0.80
C_3	0.80	0.82	0.80	0.81	0.80	0.80	0.80	0.80
C_4	0.81	0.82	0.80	0.81	0.80	0.81	0.79	0.80
Grand Mean	0.80	0.81	0.80	0.81	0.80	0.80	0.80	0.80

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.029	-
S	ns	0.041	-
M x S	ns	0.083	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Incubation temperature: 42±1 ^{0}C

Enricher

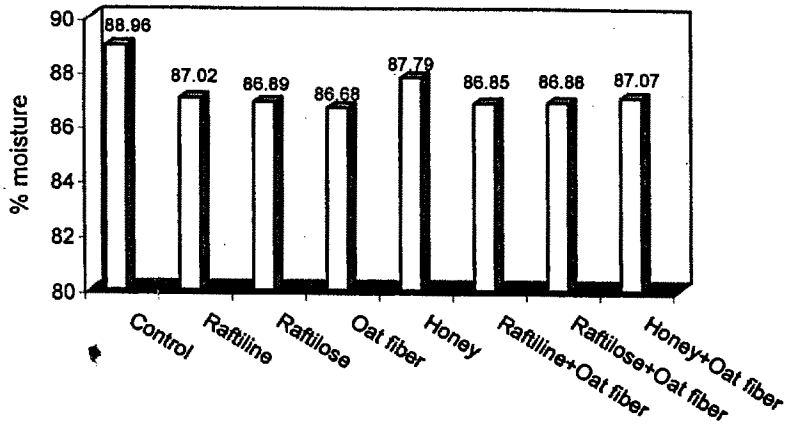

Figure 4.5 Effect of prebiotic ingredients/enricher on mean moisture content of yoghurt

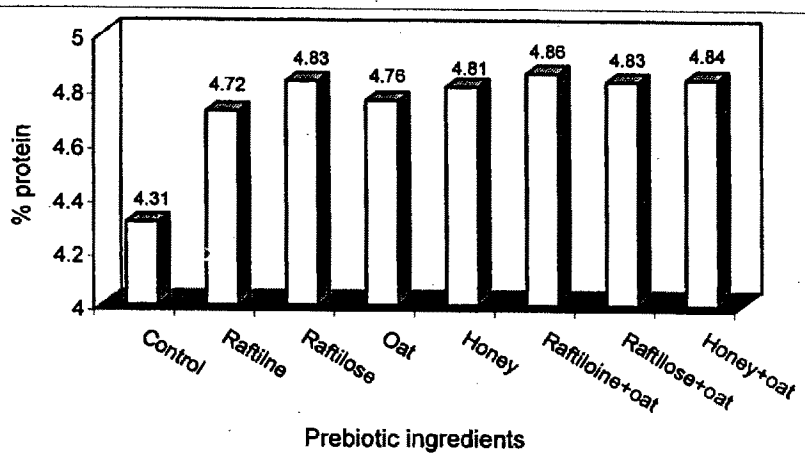

Figure 4.6 Effect of prebiotic ingredients/enricher on mean protein content of yoghurt

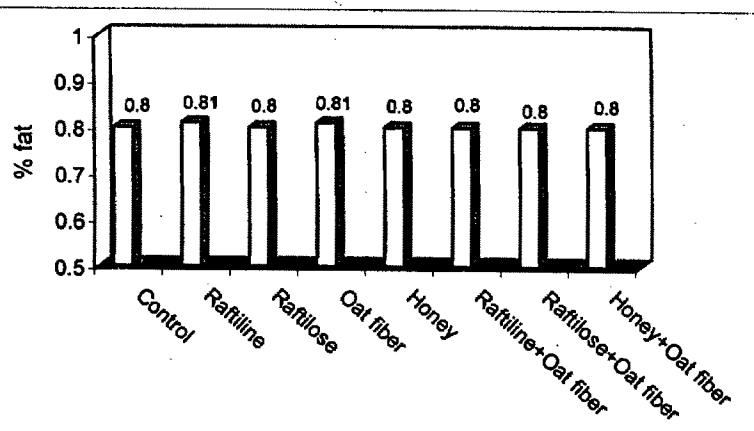

Figure 4.7 Effect of prebiotic ingredients/enricher on mean fat content of yoghurt

Tamime and Deeth (1988) have given a range of 0.84 to 4.5 percent fat in yoghurt prepared out of milk containing varying percentage of fat. **Amiri (2001)** reported one per cent fat in yoghurt prepared from cow skim milk containing 0.2 per cent fat.

4.4.1.4 Ash content

The results presented in Table 4.13 show the effect of type of bacterial culture and prebiotic ingredients along with honey on the ash content of yoghurt. Similar to protein and fat content, ash content also did not differ significantly either by preparing yoghurt using different bacterial cultures or by addition of prebiotic ingredients and/or honey in standardized milk. The ash content of the yoghurt ranged between 0.83 to 0.87 per cent. Fig. 4.8 reveals that addition of raftiline, raftilose and oat fiber to the standardized milk slightly increased the ash content of yoghurt samples. But, the addition of honey had no effect on the per cent ash of yoghurt. These differences in ash content of yoghurt were however, statistically non-significant. Slight variation in ash content of yoghurt may be attributed to variation in moisture content of yoghurt.

Tamime and Robinson (1988) have reported a little higher ash content in natural set/stirred yoghurt (1.0-1.4%) as compared to milk. This might have been due to more skim milk powder added to milk before yoghurt preparation. **Amiri (2001)** deduced 0.7 per cent ash in yoghurt prepared from cow skim milk.

Table 4.13 Effect of bacterial culture and prebiotic ingredients/enricher on the ash content of yoghurt

Bacterial Culture$(M)	% Ash in yoghurt							
	Prebiotic ingredients/enricher added (S)							
-	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4% +.5%)	Raftilose + Oat fibre (4% +.5%)	Honey + Oat fibre (3% +.5%)
C_1	0.83	0.86	0.85	0.87	0.83	0.85	0.86	0.87
C_2	0.82	0.85	0.85	0.87	0.82	0.86	0.85	0.87
C_3	0.82	0.86	0.85	0.87	0.82	0.86	0.86	0.87
C_4	0.83	0.86	0.85	0.87	0.83	0.86	0.88	0.87
Grand Mean	0.83	0.86	0.85	0.87	0.83	0.86	0.86	0.87

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.010	-
S	ns	0.015	-
M x S	ns	0.030	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Incubation temperature: 42±1 ^{0}C

Enricher

4.4.1.5 Carbohydrate content

The data presented in Table 4.14 show that the carbohydrate content of yoghurt samples differed significantly by adding prebiotic ingredients and/or honey to the standardized milk. The type of culture and interaction of culture with prebiotic ingredients, however, did not alter the carbohydrate content of yoghurt. The carbohydrate content was calculated by subtracting the sum of moisture, protein, fat and ash contents from hundred. As shown in Fig. 4.9, the carbohydrate content of yoghurt samples increased to the greatest extent by addition of oat fiber followed by raftiline, raftilose and honey at pre-optimized levels. The combination of oat fiber with raftiline, raftilose and honey showed a combined effect of both ingredients. The carbohydrate content by difference of yoghurt samples in the present investigation ranged between 5.1 to 7.0 per cent. It is notable that oat fiber, though added at minimum level (1%) amongst all ingredients, gave highest carbohydrate per cent in corresponding yoghurt. A range of 6.2-10.0 per cent lactose and other sugars has been given by **Tamime and Robinson (1988)** for sweetened and unsweetened yoghurt prepared from full fat standardized milk. **Amiri (2001)** reported 5 per cent lactose content in control yoghurt prepared from cow skim milk.

4.4.1.6 pH

The results presented in Table 4.15 reveal that the final pH of yoghurt ranged between 4.35-4.64. Only the type of bacterial culture used for fermentation affected the pH of yoghurt significantly ($P \leq 0.01$). Among the

Table 4.14 Effect of bacterial culture and prebiotic ingredients/enricher on the carbohydrate content of yoghurt

Bacterial Culture$(M)	% Carbohydrate in yoghurt							
	Prebiotic ingredients/enricher added (S)							
	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4% +.5%)	Raftilose + Oat fibre (4% +.5%)	Honey + Oat fibre (3% +.5%)
C_1	5.09	6.74	6.55	6.97	5.75	6.51	6.54	6.32
C_2	5.10	6.74	6.56	6.83	5.84	6.59	6.62	6.27
C_3	5.10	6.88	6.71	6.81	5.74	6.67	6.64	6.49
C_4	5.14	6.73	6.68	6.92	5.72	6.72	6.67	6.54
Grand Mean	5.10	6.77	6.62	6.88	5.76	6.62	6.61	6.40

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.013	-
S	**	0.018	0.072
M x S	ns	0.031	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Incubation temperature: 42±1 ^{0}C

Enricher

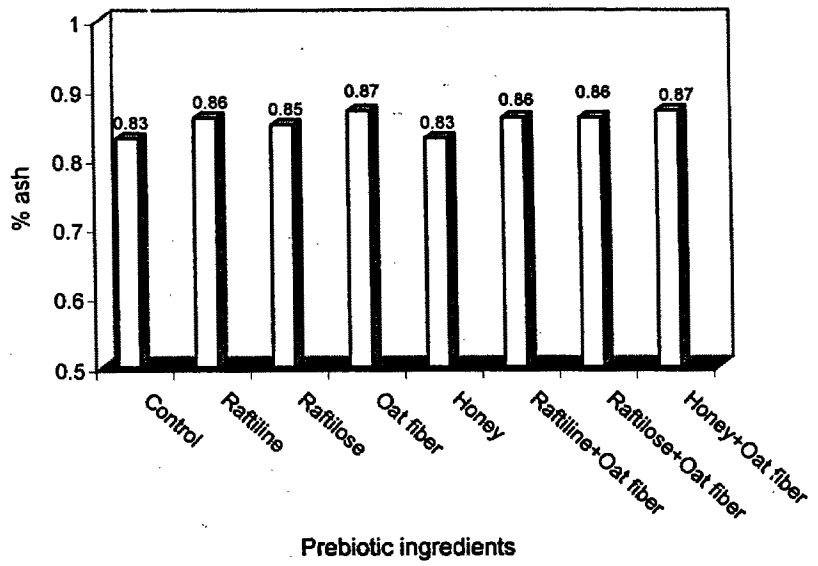

Figure 4.8 Effect of prebiotic ingredients on mean ash content of yoghurt

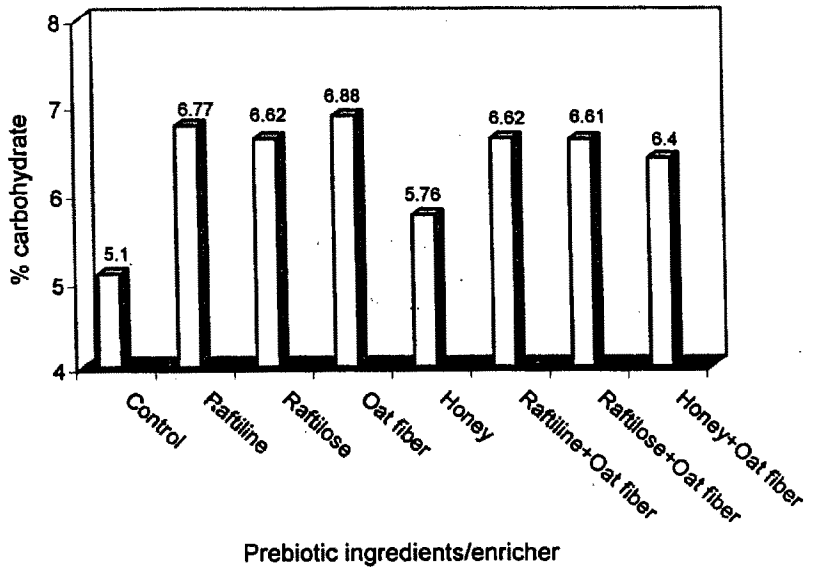

Figure 4.9 Effect of prebiotic ingredients on mean carbohydarate content of yoghurt

Table 4.15 Effect of bacterial culture and prebiotic ingredients/enricher on the pH of yoghurt

Bacterial Culture$(M)	pH of yoghurt							
	Prebiotic ingredients/enricher added (S)							
	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey (3%)	Raftiline + Oat fibre (4% +.5%)	Raftilose + Oat fibre (4% +.5%)	Honey + Oat fibre (3% +.5%)
C_1	4.58	4.57	4.58	4.57	4.58	4.58	4.57	4.58
C_2	4.40	4.38	4.39	4.39	4.38	4.38	4.38	4.39
C_3	4.63	4.64	4.63	4.64	4.63	4.64	4.64	4.63
C_4	4.37	4.36	4.36	4.35	4.35	4.35	4.35	4.35
Grand Mean	4.49	4.48	4.48	4.47	4.47	4.47	4.47	4.47

Sources of variation	F value	SEM	CD (P≤0.01)
M	**	0.013	0.050
S	ns	0.019	-
M x S	ns	0.039	-

C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*

C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*

C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*

C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Incubation temperature: 42±1 ^{0}C

Enricher

cultures (Fig 4.10), the C_4 gave yoghurt with lowest pH value (4.35), followed by C_2 (4.38), C_1 (4.58) and C_3 (4.64). It was observed that presence of *B. bifidum* in C_3 culture resulted in a high pH of yoghurt as compared to *L. acidophilus* in C_2 culture. Addition of prebiotic ingredients and/or honey and the interaction among the bacterial cultures and prebiotic ingredients had a non-significant effect on pH of yoghurt.

Similar observations were made by **Amiri (2001)** who reported that addition of *B. bifidum* in conventional yoghurt starters in the ratio of 1:1:1 resulted in a slightly higher pH of yoghurt as compared to *L. acidophilus*. Thus, the present findings are in agreement with above study. But, **Murti *et al.* (1996)** have reported that addition of *B. bifidum* caused a lowering in pH value of yoghurt. **Samona *et al.* (1996)** have noted a synergistic effect of probiotic cultures when added to yoghurt starters for fermentation. This might be the cause of minimum pH in case of yoghurt prepared by C_4 combination of culture where all 4 bacterial cultures were combined in the present investigation.

Rajagopal and Sandine (1990) and **Granata and Morr (1996)** also reported a pH range of 4.21 to 4.51 in yoghurt prepared by fermenting milk at 42^0C.

4.4.1.7 Titratable acidity

The titratable acidity (as lactic acid) of yoghurt samples ranged between 0.78 to 1.05 per cent depending upon the type of culture used (Table 4.16). The type of prebiotic ingredients and/or honey added to standardized milk had no significant effect on the titratable acidity of yoghurt samples. However, the

Table 4.16 Effect of bacterial culture and prebiotic ingredients/enricher on the titratable acidity (TA) of yoghurt

Bacterial Culture$(M)	TA (% lactic acid) of yoghurt							
	Prebiotic Ingredients/enricher added (S)							
-	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey (3%)	Raftiline + Oat fibre (4% +.5%)	Raftilose + Oat fibre (4% +.5%)	Honey + Oat fibre (3% +.5%)
C_1	0.83	0.82	0.82	0.82	0.80	0.82	0.82	0.82
C_2	0.85	0.85	0.86	0.85	0.85	0.86	0.85	0.85
C_3	0.78	0.79	0.78	0.79	0.80	0.79	0.78	0.79
C_4	0.88	0.89	0.89	0.90	0.88	0.88	0.88	0.89
Grand Mean	0.83	0.83	0.82	0.83	0.83	0.84	0.83	0.83

Sources of variation	F value	SEM	CD (P≤0.01)
M	**	0.002	0.008
S	ns	0.003	-
M x S	ns	0.006	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Incubation temperature: 42±1 ^{0}C

Enricher

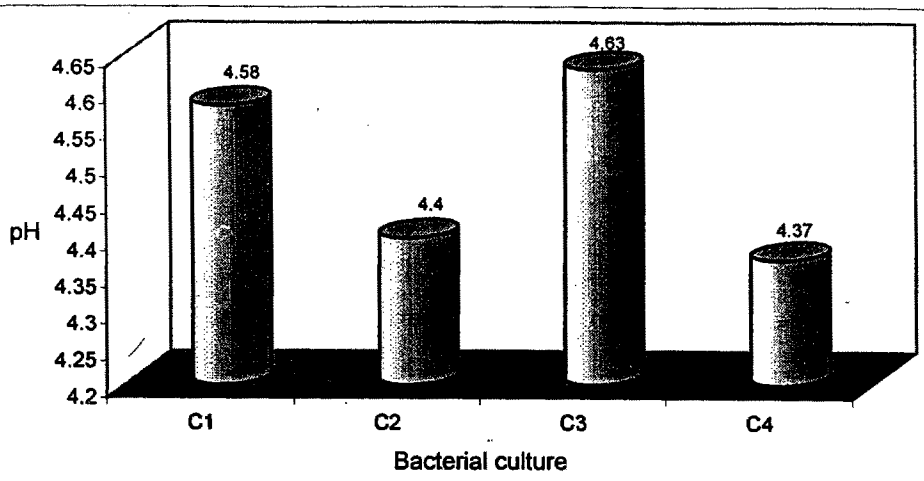

Figure 4. 10 Effect of bacterial culture on pH of yoghurt

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

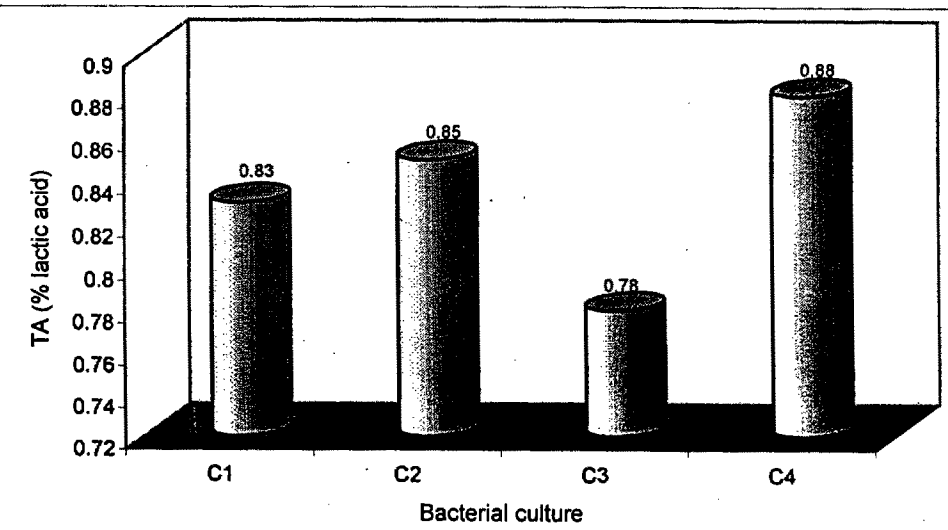

Figure 4.11 Effect of bacterial culture on titratable acidity of yoghurt

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

yoghurt samples prepared by using C_4 culture produced yoghurt with significantly ($P \leq 0.01$) higher titratable acidity (0.88%) followed by C_2 (0.86%), C_1 (0.82%) and C_3 cultures (0.79%). Similar to pH, *B. bifidum* also gave yoghurt with lower acidity as compared to *L. acidophilus* (Figure 4.11). Thus, it may be inferred that addition of *L. acidophilus* produces more lactic acid when used along with conventional yoghurt cultures for yoghurt preparation. Similar observations were also reported by **Amiri (2001)**. She found that *L. casei* (M1), *B. bifidum* (M2) and *L. acidophilus* (M3),when added to yoghurt starters individually in the ration of 1:1:1, the maximum titratable acidity was obtained in yoghurt prepared by M3 followed by one prepared by M2 and M1. **Tamime and Deeth** (1980) reported a range of 0.81-1.02 percent lactic acid in yoghurt prepared using yoghurt cultures.

4.5 Synbiotic yoghurt-cheese

4.5.1 Optimization of centrifugation speed for centrifugation of yoghurt

The control yoghurt samples were centrifuged at 5000-10,000 rpm for 15 min and the percent cheese yield were noted. The whey obtained was examined for total viable counts. The results presented in Table 4.17 revealed that the percent cheese yield increased by increasing the speed of centrifugation of yoghurt upto 9000 rpm with corresponding decrease in total viable count in whey. Centrifugation of yoghurt at speed over 9000 rpm gave a cheese with very low moisture content and mealy mouth feel. Therefore, centrifugation at 9000 rpm for 15 min was taken as optimum for yoghurt-cheese preparation.

Table 4.17 Effect of speed of centrifugation on yoghurt-cheese yield and total viable count of whey

Sl.no.	rpm	Yoghurt-cheese yield %	Total viable count of whey (cfu/ml)
1.	5000	25.34	8.5×10^5
2.	6000	25.99	7.69×10^5
3.	7000	26.43	6.21×10^5
4.	8000	27.29	9.1×10^2
5.	9000	28.60	2.1×10^2
6.	10,000	28.02	1.3×10^2

Centrifugation time: 15 min

4.5.2 Effect of bacterial culture and prebiotic ingredients/enricher addition on the physico-chemical composition of synbiotic yoghurt-cheese

The yoghurt-cheese samples were prepared by centrifugation of yoghurt at 9000 rpm for 15 min. The cheeses thus obtained were analysed for physico-chemical composition and the results obtained are presented in Table 4.18-4.24.

4.5.1.1 Moisture content

The moisture content of yoghurt-cheese ranged between 72.0 to 73.44 per cent (Table 4.18). The highest moisture content was observed in control samples prepared with out addition of any prebiotic ingredient or enricher. The type of culture had no significant effect on moisture content of yoghurt cheese. Among the prebiotic ingredients, the addition of oat fiber reduced the moisture content of yoghurt cheese samples to the maximum extent (Fig. 4.12) followed by raftiline and raftilose. Addition of honey also reduced the moisture content of yoghurt-cheese significantly ($P \leq 0.01$) but to a much lesser extent. Addition of oat fiber along with raftiline, raftilose and honey to the milk before yoghurt

Table 4.18 Effect of bacterial culture and prebiotic ingredients/enricher on the moisture content of yoghurt-cheese

Bacterial Culture$(M)	% Moisture in yoghurt-cheese							
	Prebiotic ingredients/enricher added (S)							
	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4%+ .5%)	Raftilose + Oat fibre (4% +.5%)	Honey + Oat fibre (3% +.5%)
C_1	73.44	70.98	71.08	70.56	72.77	70.86	70.59	71.40
C_2	73.39	70.97	71.05	70.20	72.82	70.56	70.48	71.31
C_3	73.34	70.97	71.13	70.43	72.78	70.53	70.46	71.29
C_4	73.39	70.97	71.11	70.48	72.89	70.57	70.43	71.24
Grand Mean	73.39	70.97	71.09	70.47	72.56	70.56	70.49	71.31

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.068	-
S	**	0.096	0.375
M x S	ns	0.193	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

preparation also reduced the moisture content of yoghurt-cheese samples. Fig. 4.12 also shows that the effect of oat fiber was much more pronounced than any other prebiotic/enriching ingredients. The interaction between bacterial cultures addition and prebiotic ingredients was non-significant.

Tamime and Robinson (1988) reported 74 per cent moisture (26% total solids) in labneh. **Salji *et al.* (1983, 1987)** reported 76-80 per cent moisture (20-24% TS) in labneh. **El-Samragy (1997)** noted 74-78 per cent moisture content (22-26% total solids) in yoghurt-cheese. The yoghurt-cheese prepared in the present investigation had comparatively higher total solids (27-30%) probably due to centrifugation and addition of skim milk powder. Slightly lower total solids (22-23%) in traditional labneh have been reported by **Beyer and Mair-waldburg (1974)**. Whey-protein concentrate incorporated labneh produced by UF milk retentate also had similar (22%) total solids (**Mahfouz *et al.*, 1992**).

4.5.1.2 Protein content

The protein content in yoghurt-cheese ranged from 17.60 to 20.11 percent (Table 4.19). The highest protein content was found in yoghurt-cheese prepared from milk containing 1 per cent oat fiber whereas the lowest protein content was found in control cheese. The differences among the cheese samples prepared from milk containing prebiotic ingredients and honey were significant ($P \leq 0.01$). On examining the Fig 4.12 and 4.13, it may be inferred that the yoghurt-cheese samples with higher moisture content had lower protein

Table 4.19 Effect of bacterial culture and prebiotic ingredients/enricher on the protein content of yoghurt-cheese

Bacterial Culture$(M)	%Protein in yoghurt-cheese							
	Prebiotic ingredients/enricher added (S)							
-	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4%+ .5%)	Raftilose + Oat fibre (4% +.5%)	Honey + Oat fibre (3% +.5%)
C_1	17.60	19.70	19.65	20.00	18.58	19.98	19.97	19.76
C_2	17.62	19.67	19.66	20.10	18.43	20.00	20.07	19.70
C_3	17.71	19.73	19.64	20.11	18.63	20.00	19.82	19.70
C_4	17.65	19.69	19.65	20.11	18.47	20.01	19.95	19.80
Grand Mean	17.64	19.69	19.65	20.08	18.55	19.99	19.96	19.74

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.063	-
S	**	0.089	0.257
M x S	ns	0.178	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

content. The moisture content of yoghurt-cheese samples actually determined the protein content of yoghurt-cheese. The effect of raftiline and raftilose incorporation in milk on the protein content of yoghurt-cheese was nearly the same. But the yoghurt-cheese samples prepared from milk after addition of 3 per cent honey had significantly ($P \leq 0.01$) lower protein content as compared to one prepared from raftiline and raftilose added milk. The yoghurt-cheese samples prepared from milk containing combinations of raftiline, raftilose and honey with oat fiber had a combined effect of two prebiotic ingredients on its protein content. Thus, it could be interpreted that addition of prebiotic ingredients caused formation of a firmer gel in yoghurt, which resulted in more retention of total solids in yoghurt-cheese and thereby proportionally higher protein. **El-Samragy (1997)** in his review has reported the protein content in the range of 8.5 to 9 per cent in labneh prepared from standardized full fat milk. **Salji *et al.* (1983, 1987)** gave a range of 9 to 11 per cent protein in labneh made from full fat milk. **Tamime and Robinson (1988)** have reported a range of 5.8 to 10.43 per cent protein in labneh made from full fat milk in different countries including Lebanon, Saudi Arabia, United Kingdom and Greece. The fat content of these labneh ranged between 6.42-11.04 per cent **(Veinoglou *et al.*, 1978)**. Thus, the fat content of milk used for labneh or yoghurt-cheese preparation has an inverse relationship with protein content in yoghurt-cheese.

4.5.2.3 Fat content

The fat content of the yoghurt-cheese ranged between 1.8 and 2.01 per cent in the present investigation. The yoghurt-cheese samples prepared by using different cultures did not differ significantly with each other with respect to their fat content (Table 4.20). However, the cheese samples prepared from milk containing raftiline, raftilose or oat fiber, singly or in combination had significantly ($P \leq 0.01$) higher fat content due to the presence of low moisture in the samples (Fig. 4.14).

Much higher fat content in commercial/traditional samples of labneh or yoghurt-cheese have been reported by many investigators as compared to one obtained in the present investigation. **Tamime (1987)** and **Tamime and Robinson (1978)** reported a range of 8.20-10.50 per cent fat in commercial labneh. **Salji *et al.* (1983, 1987)** gave lower values of 6.42-8.34 per cent fat in labneh made in Saudi Arabia. **El-Samragy (1997)** reported 9-11 per cent fat in labneh.

It is most likely that higher percent of fat in labneh/yoghurt-cheese must have been observed due to usage of full fat milk in labneh preparation. As the present study was carried out using standardized low fat (0.5%) buffalo milk, much lower value of fat in yoghurt-cheese has been observed.

4.5.2.4 Ash content

Ash content of yoghurt-cheese samples ranged between 1.05-1.13 per cent (Table 4.21). The lowest value of ash content was observed in the control

Table 4.20 Effect of bacterial culture and prebiotic ingredients/enricher on the fat content of yoghurt-cheese

Bacterial Culture$(M)	% Fat in yoghurt-cheese							
	Prebiotic ingredients/enricher added (S)							
-	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4% + .5%)	Raftilose + Oat fibre (4% +.5%)	Honey + Oat fibre (3% +.5%)
C_1	1.83	1.93	1.99	1.97	1.82	1.95	1.91	1.91
C_2	1.85	1.94	1.92	1.98	1.84	1.96	1.95	1.90
C_3	1.83	1.93	1.98	2.01	1.85	1.96	1.92	1.93
C_4	1.82	1.96	1.94	1.97	1.82	1.94	1.95	1.89
Grand Mean	1.83	1.94	1.95	1.98	1.83	1.95	1.93	1.91

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.013	-
S	**	0.019	0.076
M x S	ns	0.039	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

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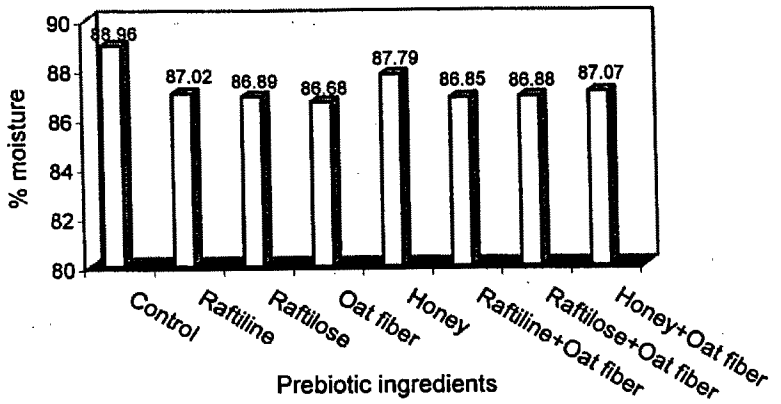

Figure 4.12 Effect of prebiotic ingredients/enricher on mean moisture content of yoghurt-cheese

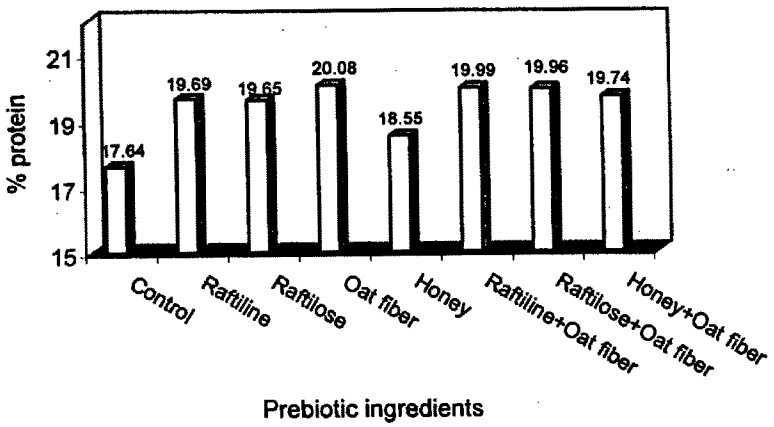

Figure 4.13 Effect of prebiotic ingredients/enricher on mean protein content of yoghurt-cheese

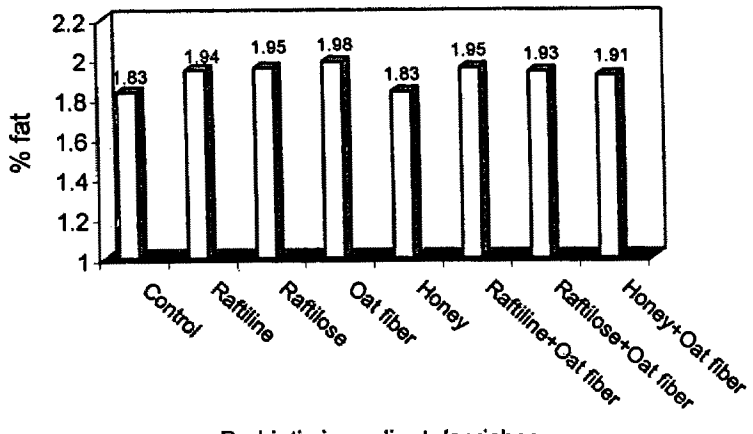

Figure 4.14 Effect of prebiotic ingredients/enricher on mean fat content of yoghurt-cheese

Table 4.21 Effect of bacterial culture and prebiotic ingredients/enricher on the ash content of yoghurt-cheese

Bacterial Culture$(M)	% Ash in yoghurt-cheese							
	Prebiotic ingredients/enricher added (S)							
	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4%+ .5%)	Raftilose + Oat fibre (4% +.5%)	Honey + Oat fibre (3% +.5%)
C_1	1.05	1.10	1.10	1.13	1.08	1.10	1.11	1.13
C_2	1.08	1.10	1.11	1.13	1.08	1.12	1.10	1.12
C_3	1.06	1.11	1.10	1.12	1.06	1.11	1.13	1.11
C_4	1.06	1.10	1.11	1.13	1.07	1.12	1.13	1.10
Grand Mean	1.06	1.10	1.10	1.12	1.07	1.11	1.11	1.11

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.008	-
S	**	0.012	0.047
M x S	ns	0.024	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

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yoghurt-cheese whereas, the highest ash content was observed in the yoghurt-cheese prepared from milk containing 1.0 per cent oat fiber. The variations between yoghurt-cheese samples prepared from milks containing raftiline and raftilose with respect to ash per cent were non-significant. The differences between ash content of control and cheese samples prepared from milk containing honey were also non-significant. However, addition of raftiline, raftilose and oat fiber to milk significantly ($P \leq 0.01$) increased the ash content of yoghurt-cheese (Fig. 4.15). The differences with respect to ash content of yoghurt-cheese prepared by using different cultures and the interaction between cultures and prebiotic ingredients were also non-significant.

Tamime (1987) and **Tamime and Robinson (1978)** have reported a range of 0.67-1.14 percent ash in experimental labneh and 1.00-1.96 per cent ash in commercial labneh samples. **Salji *et al.* (1983, 1987)** gave a range of 1.07-1.33 per cent ash in commercial labneh samples in Saudi Arabia. **El-Samragy (1997)** reported approximately 1.0 per cent salts in labneh. Thus the value of ash content of yoghurt-cheese obtained in present investigation is fairly consistent with the values reported by earlier authors.

4.5.2.5 Carbohydrate content

The carbohydrate content of yoghurt-cheese ranged between 5.68 to 6.67 per cent (Table 4.22). The effect of type of culture on the carbohydrate content of yoghurt-cheese was non-significant. However, the addition of prebiotic ingredient to milk significantly ($P \leq 0.01$) increased the carbohydrate

***Table 4.22 Effect of* bacterial culture and prebiotic ingredients/enricher on carbohydrate content yoghurt-cheese**

Bacterial Culture$(M)	% Carbohydrate in yoghurt-cheese							
	Prebiotic ingredients added (S)							
-	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4%+ .5%)	Raftilose + Oat fibre (4% +.5%)	Honey + Oat fibre (3% +.5%)
C_1	6.08	6.29	6.17	6.34	5.75	6.11	6.42	5.80
C_2	6.06	6.32	6.21	6.34	5.84	6.36	6.40	5.94
C_3	6.06	6.26	6.15	6.33	5.68	6.46	6.67	6.01
C_4	6.08	6.30	6.19	6.31	5.75	6.34	6.54	5.97
Grand Mean	6.07	6.29	6.18	6.33	5.75	6.31	6.50	5.93

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.013	-
S	**	0.018	0.072
M x S	ns	0.037	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

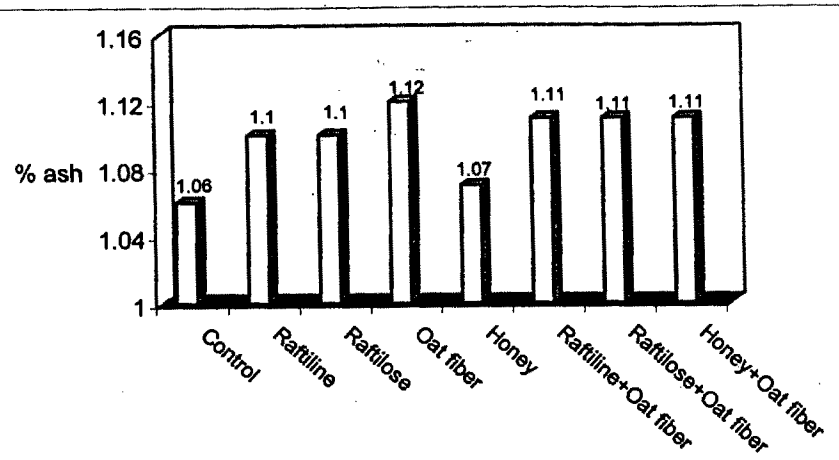

Figure 4.15 Effect of prebiotic ingredients on mean ash content of yoghurt-cheese

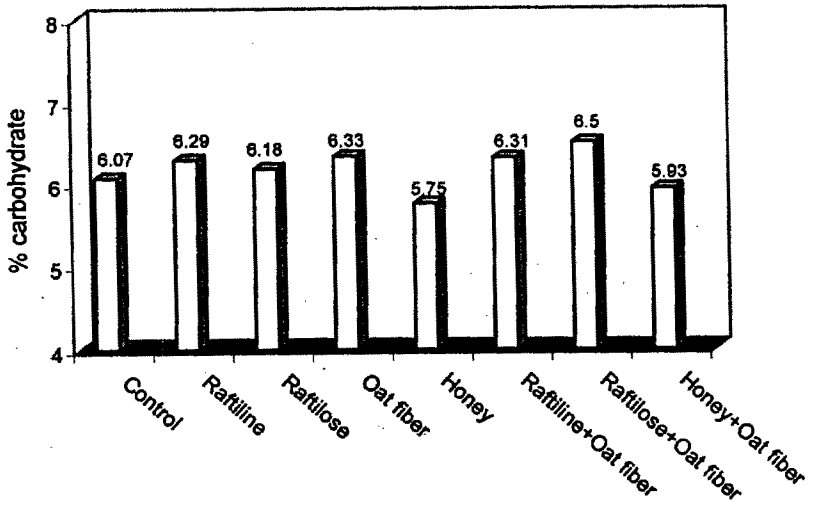

Figure 4.16 Effect of prebiotic ingredients on mean carbohydarate content of yoghurt-cheese

content of yoghurt-cheese samples. The cheese sample prepared after adding 3 per cent addition of honey to the milk gave yoghurt cheese with significantly ($P \leq 0.01$) lower content of carbohydrate. Such an observation was probably made due to the loss of honey sugars in whey and higher moisture content in yoghurt-cheese (Fig 4.16). The combination of raftilose and oat fiber gave the yoghurt cheese with highest carbohydrate content.

Tamime (1987) and **Tamime and Robinson (1978)** have reported 3.31-5.10 per cent lactose in labneh produced in United Kingdom. **Salji *et al.* (1983, 1987)** has reported 2.86-4.91 per cent lactose (calculated by difference) in labneh prepared in Saudi Arabia, whereas **El-Samragy (1997)** gave a range of 3.5-4 per cent carbohydrate in labneh or yoghurt-cheese. The higher values of carbohydrate content obtained in the present investigation might have been a result of low fat content (0.5%) in milk used for yoghurt-cheese preparation. which led to higher protein and carbohydrate content in yoghurt-cheese having a total solids content fairly agreeing with reported works on chemical composition by various authors.

4.5.2.6 pH

The data presented in Table 4.23 show that addition of prebiotic ingredients with or without honey had no significant effect on the pH of yoghurt-cheese. However, the culture used in the preparation of yoghurt-cheese significantly ($P \leq 0.01$) altered its pH. The pH of yoghurt-cheese samples ranged between 4.23-4.47. The lowest pH was obtained in the yoghurt-cheese

samples prepared by using C_4 combination of starter cultures followed by C_2, C_1, and C_3 cultures (Fig. 4.17). The pH of yoghurt-cheese samples was slightly lower as compared to that of yoghurt due to some production of lactic acid during time lapse between centrifugation and removal of whey from curd. The work done by **El-Samragy (1997)** has reported even lower pH value for labneh or yoghurt-cheese as inferred by a higher lactic acid range of 1.6-2.5 per cent in the same.

4.5.2.7 Titratable acidity

Like pH, the effect of bacterial culture used for yoghurt-cheese preparation was significant ($P \leq 0.01$) on the titratable acidity of yoghurt-cheese (Table 4.24). But the prebiotic ingredients and honey incorporation in milk did not alter the acidity of cheese significantly. The interactions between the type of cultures and prebiotic ingredients or honey were also found to be non-significant. It may be seen from Fig 4.18 that the C_4 culture gave the highest titratable acidity followed by C_2, C_1 and C_3. On comparing Fig. 4.17 and 4.18, it is revealed that there is an obvious inverse relationship between pH and titratable acidity.

El-Samragy (1997) has reported a higher range of 1.6-2.5 per cent lactic acid in labneh or yoghurt-cheese. The traditional method of labneh preparation involves longer time for whey removal by drainage from the cloth bag and thus a corresponding increase in acidity takes place.

Table 4.24 Effect of bacterial culture and prebiotic ingredients/enricher on Titratable acidity of yoghurt-cheese

Bacterial Culture$(M)	% TA (lactic acid)							
	Prebiotic ingredients/enricher added (S)							
	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4%+ .5%)	Raftilose + Oat fibre (4% +.5%)	Honey + Oat fibre (3% +.5%)
C_1	1.10	1.11	1.12	1.11	1.10	1.12	1.11	1.10
C_2	1.17	1.16	1.15	1.17	1.16	1.15	1.15	1.16
C_3	1.03	1.03	1.04	1.04	1.04	1.03	1.03	1.04
C_4	1.19	1.20	1.20	1.20	1.19	1.19	1.19	1.20
Grand Mean	1.12	1.12	1.13	1.13	1.12	1.13	1.12	1.12

Sources of variation	F value	SEM	CD (P≤0.01)
M	**	0.002	0.015
S	ns	0.008	-
M x S	ns	0.017	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

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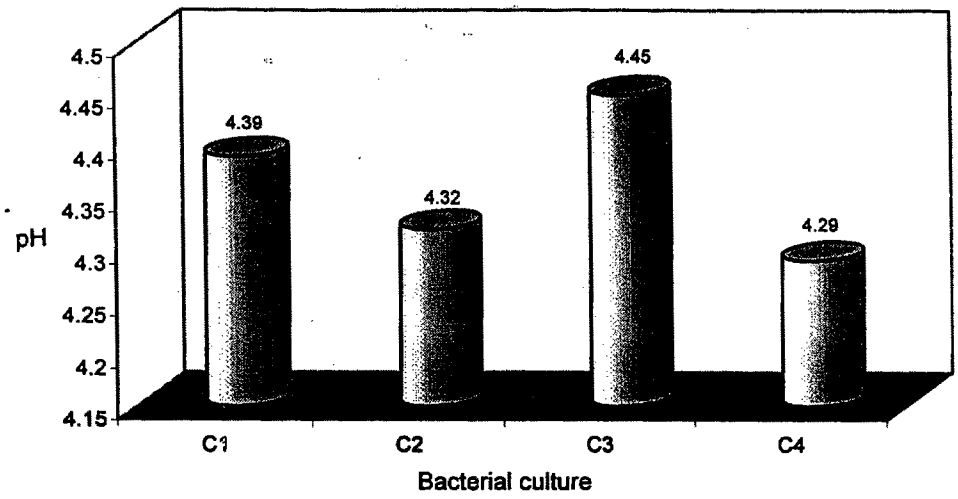

Figure 4. 17 Effect of bacterial culture on pH of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

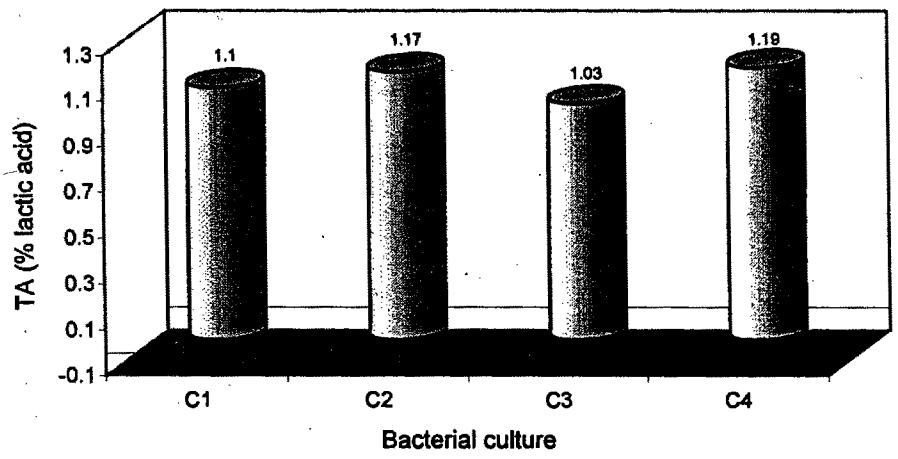

Figure 4.18 Effect of bacterial culture on titratable acidity of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

4.6 Effect of starter culture and prebiotic ingredients/ enricher addition on physico-chemical composition of whey

Yoghurt prepared from standardized milk containing different prebiotic ingredients and enricher (honey) and fermented by using four different sets of culture combinations were centrifuged at 9000 rpm for 15 min and the whey obtained after centrifugation was analysed for physico-chemical characteristics. The data obtained are presented in Table 4.25-4.31.

4.6.1 Moisture content

The whey obtained by centrifugation of yoghurt contained approximately 7.5-9.0 per cent total solids. The moisture content in the control samples was lowest and did not significantly differ with whey obtained from yoghurt prepared after adding honey to standardized milk (Table 4.25). The differences in the moisture content of whey obtained after centrifugation of yoghurt prepared from standardized milk containing raftiline, raftilose or oat fiber or their combinations were also non-significant. The whey obtained from control yoghurt and that obtained from yoghurt prepared after addition of honey had appreciably lower moisture content as compared to other treatments (Fig. 4.19). However, the differences were statistically non-significant.

4.6.2 Protein content

The average protein content of whey samples ranged from 1.31 to 1.42 per cent. The protein content in whey samples remained unaltered by using different culture combinations for fermentation. However, the addition of

Table 4.25 Effect of bacterial culture and prebiotic ingredients/enricher on the moisture content of yoghurt-cheese whey

Bacterial Culture$(M)	% Moisture in yoghurt-cheese whey							
	Prebiotic ingredients/enricher added (S)							
	Control	Raftiline	Raftilose	Oat fiber	Honey#	Raftiline + Oat fibre	Raftilose + Oat fibre	Honey + Oat fibre
-	-	(4%)	(4%)	(1%)	(3%)	(4%+.5%)	(4%+.5%)	(3% +.5%)
C_1	91.20	92.70	92.12	92.89	91.97	92.58	92.48	92.28
C_2	91.30	92.69	92.01	92.99	91.95	92.01	92.49	92.15
C_3	91.17	92.04	92.26	92.80	91.89	92.51	92.47	92.09
C_4	91.09	92.68	92.60	92.79	91.90	92.46	92.48	92.28
Grand Mean	91.19	92.53	92.50	92.86	91.92	92.46	92.50	92.20

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.308	-
S	ns	0.435	-
M x S	ns	0.817	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

Table 4.26 Effect of bacterial culture and prebiotic ingredients/enricher on the protein content of yoghurt-cheese whey

Bacterial Culture$(M)	% Protein in yoghurt-cheese whey							
	Prebiotic ingredients/enricher added (S)							
	Control	Raftiline	Raftilose	Oat fiber	Honey#	Raftiline + Oat fibre	Raftilose + Oat fibre	Honey + Oat fibre
-	-	(4%)	(4%)	(1%)	(3%)	(4%+.5%)	(4%+.5%)	(3% +.5%)
C_1	1.32	1.40	1.41	1.38	1.33	1.35	1.40	1.40
C_2	1.31	1.40	1.41	1.41	1.35	1.45	1.40	1.42
C_3	1.32	1.40	1.41	1.42	1.42	1.35	1.42	1.43
C_4	1.31	1.41	1.42	1.42	1.33	1.42	1.42	1.42
Grand Mean	1.31	1.40	1.41	1.41	1.34	1.41	1.41	1.42

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.007	-
S	**	0.012	0.042
M x S	ns	0.022	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

different prebiotic ingredients and enricher caused a significant ($P \leq 0.01$) variation in the protein content of whey obtained during preparation of yoghurt-cheese (Table 4.26). The lowest protein content was obtained in control whey samples followed by whey obtained from honey added yoghurt. The addition of raftiline, raftilose and oat fiber to standardized milk either singly or in combination for yoghurt preparation increased the protein content of whey significantly ($P \leq 0.01$) (Fig. 4.20). However, the effects of interactions between the type of culture and prebiotic ingredients added to milk were non-significant.

4.6.3 Fat content

The effect of type of culture and prebiotic ingredient added to milk on the fat content of whey obtained during yoghurt-cheese preparation was non-significant (Table 4.27). All the whey samples contained approximately 0.1 per cent fat (Fig. 4.21).

4.6.4 Ash content

Ash content of whey obtained during preparation of yoghurt-cheese was in the range of 0.07-0.08 per cent (Table 4.28). The type of starter culture used for fermentation had non-significant effect on the ash content of whey. Some variation may be seen from the Fig. 4.22 but these differences were not statistically significant. The difference in ash content may be attributed to the variations in replications.

Table 4.27 Effect of bacterial culture and prebiotic ingredients/enricher on the fat content of yoghurt-cheese whey

Bacterial Culture$(M)	% Fat content in yoghurt-cheese whey							
	Prebiotic ingredients/enricher added (S)							
	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4%+.5%)	Raftilose + Oat fibre (4%+.5%)	Honey + Oat fibre (3% +.5%)
C_1	0.09	0.10	0.09	0.10	0.10	0.10	0.09	0.10
C_2	0.10	0.10	0.09	0.10	0.10	0.09	0.09	0.10
C_3	0.10	0.10	0.10	0.10	0.09	0.09	0.10	0.10
C_4	0.10	0.10	0.10	0.10	0.09	0.10	0.10	0.10
Grand Mean	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.003	-
S	ns	0.004	-
M x S	ns	0.009	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

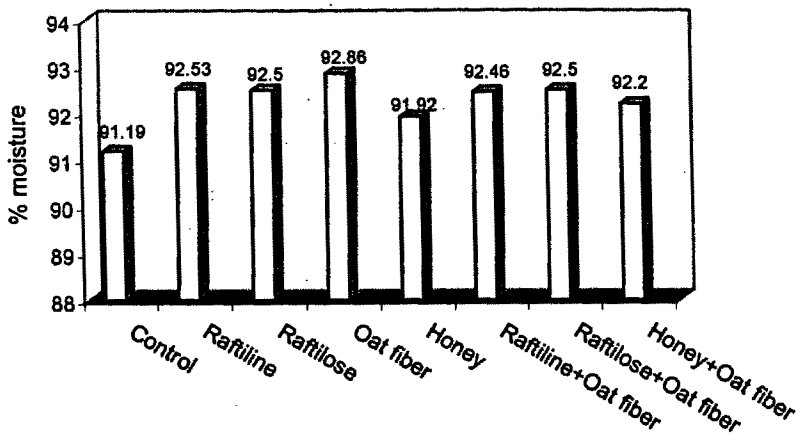

Figure 4.19 Effect of prebiotic ingredients/enricher on mean moisture content of whey

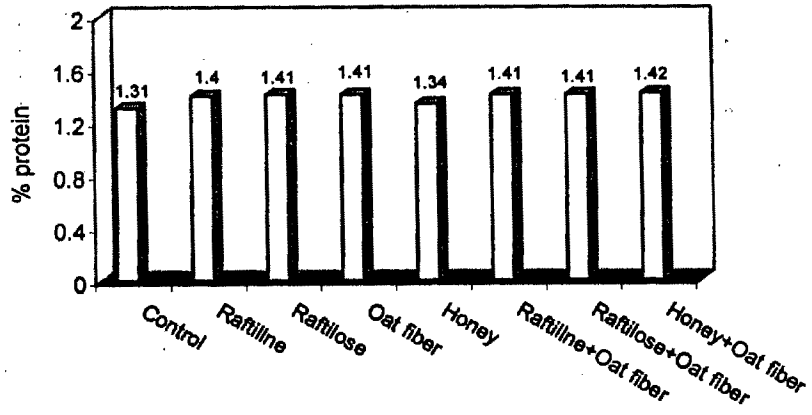

Figure 4.20 Effect of prebiotic ingredients/enricher on mean protein content of whey

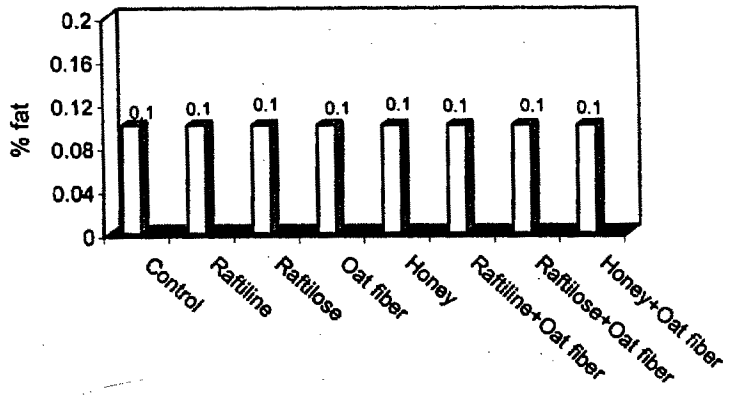

Figure 4.21 Effect of prebiotic ingredients/enricher on mean fat content of whey

Table 4.28 Effect of bacterial culture and prebiotic ingredients/enricher on the ash content of yoghurt-cheese whey

Bacterial Culture$(M)	% Ash in yoghurt-cheese whey							
	Prebiotic ingredients/enricher added (S)							
	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + oat fibre (4%+.5%)	Raftilose + Oat fibre (4%+.5%)	Honey + Oat fibre (3% +.5%)
C_1	0.09	0.08	0.07	0.07	0.08	0.08	0.08	0.09
C_2	0.09	0.08	0.09	0.09	0.08	0.07	0.07	0.08
C_3	0.08	0.07	0.09	0.09	0.08	0.08	0.07	0.09
C_4	0.08	0.07	0.08	0.09	0.08	0.08	0.07	0.08
Grand Mean	0.08	0.07	0.08	0.08	0.07	0.07	0.07	0.08

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.021	-
S	ns	0.029	-
M x S	ns	0.059	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

4.6.5 Carbohydrate content

The carbohydrate content of the yoghurt-cheese whey ranged from 4.37 to 7.42 per cent. The carbohydrate content of whey samples differed significantly ($P \leq 0.01$) by adding different prebiotic ingredient and honey to milk. However, the effect of type of culture on carbohydrate content of whey was found non-significant (Table 4.29). It may be seen from Fig. 4.23 that the highest carbohydrate content was found in whey obtained from control samples followed by honey, raftilose, raftiline and oat fiber added samples of yoghurt-cheese singly. But, when ingredients were added to milk combination of, highest carbohydrate content was obtained in combination of honey with oat fiber followed by combinations of raftiline and raftilose with oat fiber. Such differences might have been due to the variation in total solids content in whey obtained from different set of treatments.

4.6.6 pH

The pH of whey samples obtained during preparation of yoghurt-cheese ranged between 4.03-4.25 (Table 4.30). The type of culture used for yoghurt-cheese preparation affected the pH of whey significantly ($P \leq 0.01$). However, the effects of prebiotic ingredients and honey addition to standardized milk before fermentation on pH values of whey were non-significant. The highest pH was observed in whey obtained by use of C_4 culture followed by C_2, C_1 and C_3 cultures (Fig. 4.24). The results obtained indicate that the trend of pH of whey remained similar to that of yoghurt and yoghurt-cheese samples.

Table 4.29 Effect of bacterial culture and prebiotic ingredients/enricher on the carbohydrate content of yoghurt-cheese whey

Bacterial Culture$(M)	%Carbohydrate in yoghurt-cheese whey							
	Prebiotic ingredients/enricher added (S)							
-	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4%+.5%)	Raftilose + Oat fibre (4%+.5%)	Honey + Oat fibre (3% +.5%)
C_1	6.30	5.71	6.31	5.56	6.52	5.89	5.95	6.13
C_2	7.20	5.73	6.20	5.41	6.52	6.37	5.95	6.24
C_3	7.33	6.19	6.14	5.60	6.52	5.98	5.94	6.30
C_4	7.42	5.74	5.80	5.60	6.60	5.94	5.93	6.12
Grand Mean	7.31	5.93	6.10	5.54	6.54	6.04	5.94	6.19

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.011	-
S	**	0.015	0.597
M x S	ns	0.031	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

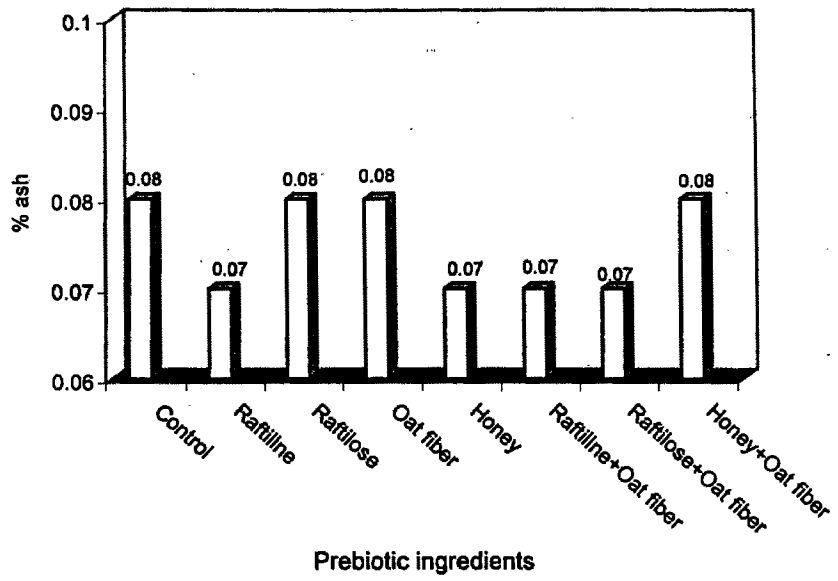

Figure 4.22 Effect of prebiotic ingredients on mean ash content of whey

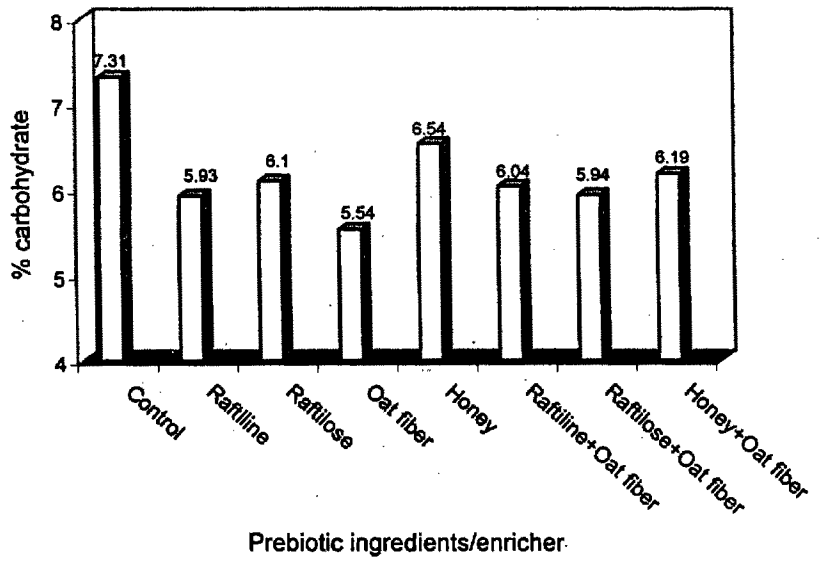

Figure 4.23 Effect of prebiotic ingredients on mean carbohydarate content ofwhey

Table 4.30 Effect of bacterial culture and prebiotic ingredients/enricher on pH of yoghurt-cheese whey

Bacterial Culture$(M)	pH of yoghurt-cheese whey							
	Prebiotic ingredients/enricher added (S)							
-	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + Oat fibre (4%+.5%)	Raftilose + Oat fibre (4%+.5%)	Honey + Oat fibre (3% +.5%)
C_1	4.21	4.20	4.21	4.21	4.21	4.21	4.21	4.21
C_2	4.15	4.16	4.15	4.16	4.15	4.17	4.15	4.16
C_3	4.24	4.25	4.24	4.23	4.24	4.24	4.24	4.24
C_4	4.13	4.13	4.13	4.14	4.14	4.14	4.14	4.13
Grand Mean	4.18	4.19	4.18	4.18	4.19	4.19	4.18	4.18

Sources of variation	F value	SEM	CD (P≤0.01)
M	**	0.002	0.009
S	ns	0.003	-
M*S	ns	0.006	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

Table 4.31 Effect of bacterial culture and prebiotic ingredients/enricher on titratable acidity (TA) of yoghurt-cheese whey

Bacterial Culture*(M)	TA (% lactic acid) of yoghurt-cheese whey							
	Prebiotic ingredients/enricher added (S)							
-	Control -	Raftiline (4%)	Raftilose (4%)	Oat fiber (1%)	Honey# (3%)	Raftiline + oat fibre (4%+.5%)	Raftilose + Oat fibre (4%+.5%)	Honey + Oat fibre (3% +.5%)
C_1	1.68	1.70	1.69	1.70	1.69	1.70	1.69	1.70
C_2	1.74	1.75	1.74	1.75	1.75	1.75	1.74	1.75
C_3	1.65	1.64	1.64	1.63	1.64	1.64	1.65	1.65
C_4	1.76	1.77	1.76	1.77	1.76	1.77	1.76	1.76
Grand Mean	1.70	1.71	1.70	1.71	1.71	1.71	1.70	1.771

Sources of variation	F value	SEM	CD (P≤0.01)
M	**	0.004	0.018
S	ns	0.006	-
M x S	ns	0.013	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

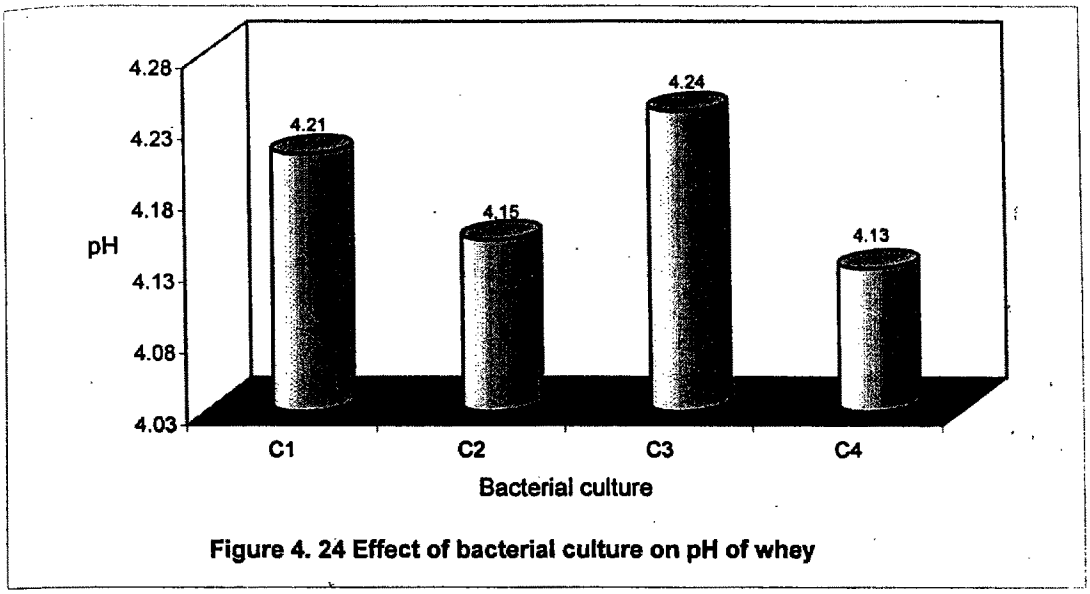

Figure 4. 24 Effect of bacterial culture on pH of whey

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

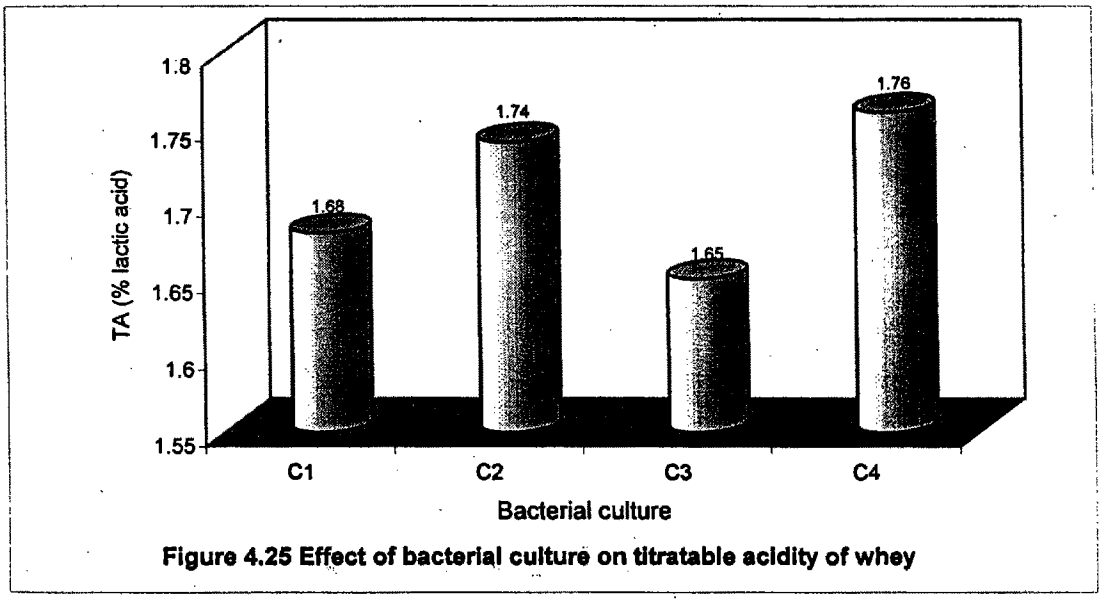

Figure 4.25 Effect of bacterial culture on titratable acidity of whey

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

4.6.7 Titratable acidity

The titratable acidity had inverse relationship with pH and was also affected significantly ($P \leq 0.01$) by type of culture used for fermentation of milk. However, it remained unaltered by addition of different prebiotic ingredients and/or enricher (Table 4.31). The highest titratable acidity was observed in whey obtained by fermenting milk using C_4 culture followed by C_2, C_1 and C_3 (Fig. 4.25).

4.7 Biocompatibility studies of bacterial cultures

All the four combinations of bacterial cultures namely C_1 (control), C_2, C_3 and C_4 used for the yoghurt preparation were subjected to biocompatibility studies which included changes in pH and titratable acidity during incubation for the preparation of yoghurt cheese, bile tolerance studies at 0.0, 0.3 and 0.5 per cent sodium glycolate in lactic broth and antagonistic activities towards selected human pathogens.

4.7.1 Changes in pH

The standardized buffalo milk containing 0.5 per cent fat and 2 per cent skim milk powder was inoculated separately using four different sets of starter cultures. The inoculated milk was incubated for four hours at 42±1°C to obtain yoghurt and after centrifugation, the yoghurt-cheese was again incubated upto 16 hours at the same temperature. The initial pH of milk ranged from 6.15 to 6.16 after inoculation depending upon the type of culture added. After 4 h, pH of yoghurt obtained ranged between 4.28-4.44 and finally after 16 h, the final

Table 4.32 Changes in pH during incubation of yoghurt and yoghurt-cheese prepared by different bacterial cultures

Bacterial culture$ (M)	pH				
	Incubation time in hours (S)				
	0	4	8	12	16
C_1	6.16	4.40	4.12	3.60	3.19
C_2	6.15	4.30	4.10	3.49	3.12
C_3	6.15	4.44	4.17	3.62	3.22
C_4	6.15	4.28	4.07	3.46	3.10
Mean	6.15	4.35	4.11	3.54	3.15

Source of variation	F value	SEM	CD (P<0.01)
M	**	0.005	0.402
S	**	0.006	0.024
M x S	**	0.013	0.049

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Incubation temperature: 42±1^{0}C

pH of cheese ranged between 3.10 and 3.22 (Table 4.32). The incubation time as well as the type of starter culture affected the pH significantly ($P \leq 0.01$). It may be seen from Fig 4.26 that a steep reduction in pH took place during the first four hours of incubation during which the milk was set to yoghurt, followed by a slower decrease in pH up to 8 h and then a steady fall in pH up to 16 h of incubation of yoghurt-cheese samples prepared by using all the 4 starter cultures. Among the different cultures, the minimum final pH was obtained in case of cheese prepared by C_4 culture followed by C_2, C_1 and C_3 cultures. The slower decrease in the fall in pH may be attributed probably due to effect of lower pH values, which inhibited the bacterial activity at the final phase of incubation. During 4-8 h, the yoghurt was centrifuged and the cheese prepared was then incubated again, therefore, the culture took some time to become again fully active, thus resulting in a slower decrease in pH value. The interpretation of the current findings with regards to work done by various authors has been discussed in section 4.7.2.

4.7.2 Changes in titratable acidity

The titratable acidity increased during incubation of milk for the setting to yoghurt upto 4 h, centrifugation of yoghurt for yoghurt-cheese preparation and incubation of cheese upto a total of 16 h. There was an inverse relationship between pH and titratable acidity (Table 4.33). The effect of type of culture as well as incubation time affected the titratable acidity significantly ($P \leq 0.01$). The interactions between the starter culture and incubation time was also

Table 4.33 Changes in titratable acidity during incubation of yoghurt and yoghurt-cheese prepared by different bacterial cultures

Bacterial culture$ (M)	TA (% lactic acid)				
	Incubation time in hours (S)				
	0	4	8	12	16
C_1	0.34	1.08	1.35	1.57	1.80
C_2	0.33	1.13	1.40	1.63	1.82
C_3	0.34	1.02	1.29	1.51	1.78
C_4	0.34	1.15	1.42	1.64	1.84
Mean	0.33	1.16	1.36	1.58	1.81

Source of variation	F value	SEM	CD (P<0.01)
M	**	0.004	0.017
S	**	0.005	0.019
M x S	**	0.001	0.038

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Incubation temperature: 42±1^{0}C

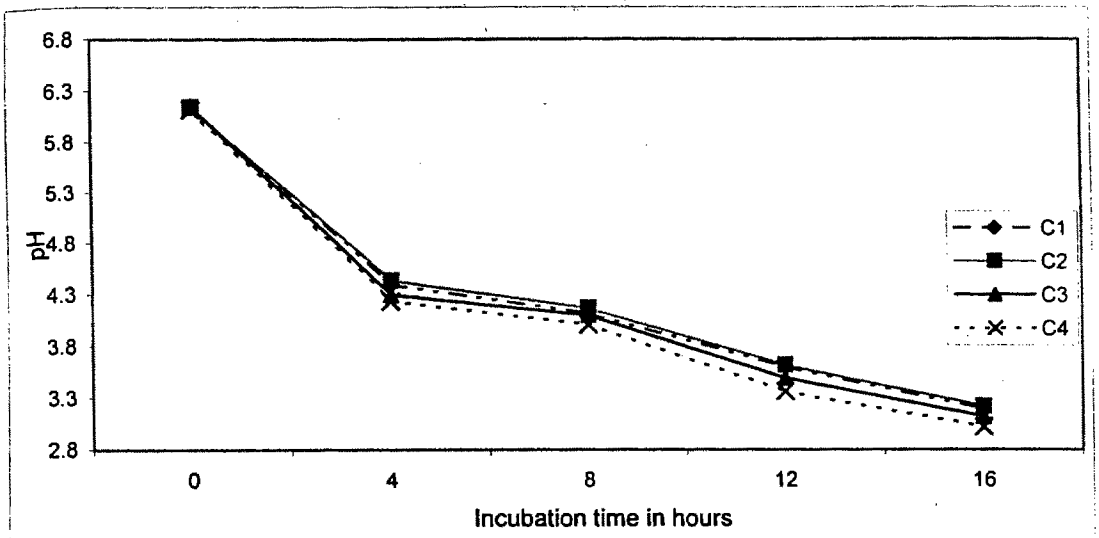

Figure 4.26 Effect of bacterial culture combination and incubation time on pH of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

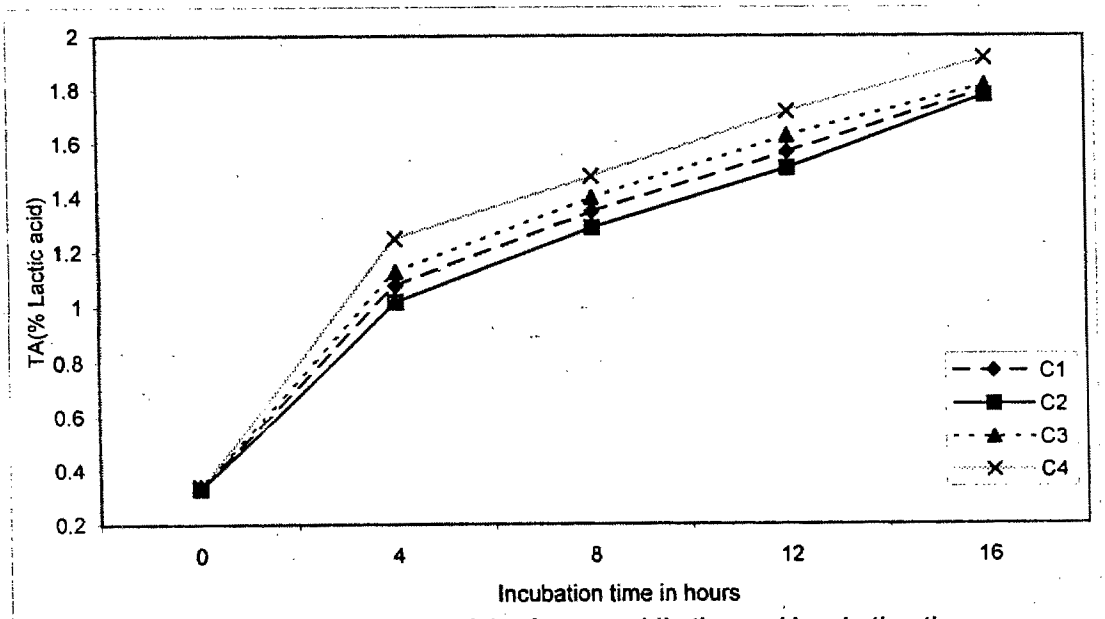

Figure 4.27 Effect of bacterial culture combibation and incubation time on titratable acidity of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

significant ($P \leq 0.01$). After inoculation, the milk had 0.33-0.34 per cent acidity, which was increased to 1.02-1.15 per cent when the milk was set to yoghurt, and the final titratable acidity of yoghurt-cheese prepared out of the yoghurt ranged between 1.78 and 1.84 per cent at the end of 16 h of incubation. It may be seen from Fig. 4.27 that the highest titratable acidity was attained in case of C_4 culture, followed by C_2, C_1 and C_3 cultures The trends obtained in the decrease of pH and increase in titratable acidity was same. A steep increase in titratable acidity was observed during first four hours of incubation period during which the milk was set to yoghurt followed by a gradual but steady increase in titratable acidity during incubation of yoghurt cheese.

Amiri (2001) reported that addition of *L. casei* or *L. acidophilus* to yoghurt culture in 1:1:1 ratio resulted in slight decrease and increase of pH and titratable acidity of yoghurt, respectively. She further reported that incorporation of *B. bifidum* in yoghurt starter at the same level led to slight increase in pH and a slight decrease in titratable acidity of yoghurt. Thus, the present study is in confirmation to her findings. She also found that pH and titratable acidity of yoghurt continuously decreased and increased, respectively, during the 16 h of incubation.

Rajagopal and Sandine (1990) and **Granata and Morr (1996)** reported that the pH and titratable acidity (% lactic acid) for yoghurt samples using yoghurt culture for 4 h of incubation at 42°C ranged from 4.24 to 4.51 and from 0.86 to 1.03 per cent, respectively. In the present investigation, pH

obtained after 4 hrs of incubation fall within the above reported range. **Murti *et al.* (1993)** reported that yoghurt samples prepared by yoghurt culture (Y) and yoghurt culture with *B. bifidum* (YB) exhibited 5-6 hours incubation to reach pH 4 or lower and produced upto 1.3 per cent acidity. They concluded that there was no difference in the final acidity of Y and YB. But YB culture took longer time of incubation to reach the same acidity level. In contrary to above findings, a slight increase in pH and a slight decrease in acidity have been met with when *B. bifidum* was used along with yoghurt culture in the present investigation.

Somona *et al.* (1996) described that the levels of acid in mixed yoghurt and Bifidobacterium cultures were a reflection of the combination of the two, which suggested that there was a degree of interference between the cultures. Thus our findings are in consonance with this study. The present investigation also revealed that when both *L. acidophilus* and *B. bifidum* are added to yoghurt culture in 1:1:1:1 ratio (C_4) an increased synergistic effect was obtained resulting in maximum acidity or minimum pH in the yoghurt.

4.7.3 Bile tolerance

Yoghurt cultures with and without probiotic bacteria namely *L. acidophilus* and *B. bifidum* were inoculated in lactic broth containing 0.0, 0.3 and 0.5 per cent bile salt (sodium glycolate) and the broths were incubated at 37±1°C for 16 h. The optical density was measured at an interval of 4 h during incubation period. The per cent growth inhibited at every stage of incubation

Table 4.34 Growth inhibition of yoghurt-cheese cultures by bile salt in lactic broth

Bacterial culture$ (M)	% Growth inhibition									
	Incubation time in hours (S)									
	0		4		8		12		16	
	% Bile salt (SS)									
	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5	0.3	0.5
C_1	91.6	96	96	97.1	97.9	98	99	99	100	100
C_2	68.1	79.1	55.1	82.1	57.1	86.2	61	89	63	90
C_3	52.1	70	55.1	72.1	56	74	60	75	64	70
C_4	46.1	58	49.1	80	50	64	52	67	60	68

Sources of variation	F value	SEM	CD (P<0.05)
M	**	0.004	0.011
S	**	0.004	0.012
SS	**	0.003	0.009
M x S	**	0.009	0.025
S x SS	**	0.007	0.019
M x SS	**	0.008	0.021
M x S x SS	**	0.015	0.043

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

(Bile salt): Sodium glycolate
Growth medium: Lactic broth
Incubation temperature: 37±1^{0}C

$$\% \text{ Growth inhibition} = \frac{X-Y}{Y} \times 100,$$

where, X = Optical density of lactic broth with out bile salt
Y = Optical density of lactic broth with 0.3 or 0.5 % bile salt

was calculated and the results obtained are presented in Table 4.34. As the concentration of bile salt was increased, the per cent inhibition of culture growth also increased significantly ($P \leq 0.01$) during the incubation. The different set of starter cultures had significantly ($P \leq 0.01$) different bile tolerance capacity. The effect of bile on per cent inhibition of bacterial growth was also incubation time dependent. As the incubation time increased, the per cent inhibition of culture increased.

Just after adding 0.3 per cent bile salt in the broth, the maximum inhibition of culture growth occurred in C_1 culture followed by C_2, C_3 and C_4 cultures (Fig. 4.28) and after 4 h of incubation, the inhibition of C_1 culture was found as high as 96 per cent followed by 55.1 per cent in case of C_2 and C_3 and 49.1 per cent for C_4 cultures. Later on, a gradual increase in percent growth inhibited was observed in all cultures. It was also found that the growth of C_1 (control) culture was inhibited to the extent of 100 per cent after 16 h of incubation when 0.3 per cent bile salt concentration was added to broth. But when probiotic bacteria was added to control culture (C_2, C_3 & C_4), per cent growth inhibited was much less, thus indicating higher bile tolerance of probiotic bacteria. Among the probiotic bacteria added culture combinations, C_4 having both *L. acidophilus* and *B. bifidum* was most tolerant to bile (maximum growth inhibition was to the extent of 60 per cent at the end of incubation) followed by C_2 (63%) and C_3 (64%). It was also noted that per cent inhibition of growth of C_2 culture decreased between 0-4 hours of

incubation and then increased gradually (Fig. 4.28). However, no explanation was available for this observation.

When 0.5 per cent bile salt was added to lactic broth, 96 per cent growth was inhibited in case of control culture (C_1) just after addition of bile salt (at 0 hr of incubation) and nearly complete inhibition resulted in next 4 h of incubation. The growth inhibition $C_1$was 98, 99 and 100 per cent at the end of 9, 12 and 16 h of incubation, respectively. In case of C_2, C_3 and C_4 cultures which had probiotic bacteria, the inhibition of growth of the culture by bile was not more than 90 per cent (C_2) even after 16 h of incubation. The least inhibition was in case of C_4 culture (68%), closely followed by C_3 (70%) at the end of incubation period (Fig. 4.29). It was also observed that in case of C_3 and C_4 cultures, the per cent growth inhibition decreased after 12 h and 4 h of incubation, respectively. After 8 h of incubation, the per cent growth inhibition in case of C_4 cultures increased gradually and steadily.

Thus from the above observations, it may be concluded that addition of probiotic bacteria to yoghurt starter appreciably increased the bile tolerance capacity and that probably synergistic effect of 2 probiotic bacteria namely *L. acidophilus* and *B. bifidum* with yoghurt culture was also met with leading to higher bile tolerance capacity of mixed cultures. It is revealed from Fig. 4.28 that control culture (C_1) couldn't sustain 0.3 per cent bile salt but addition of probiotic bacteria to control culture could make the culture combinations namely C_2, C_3 and C_4 tolerant even to 0.5 per cent bile salt in the lactic broth.

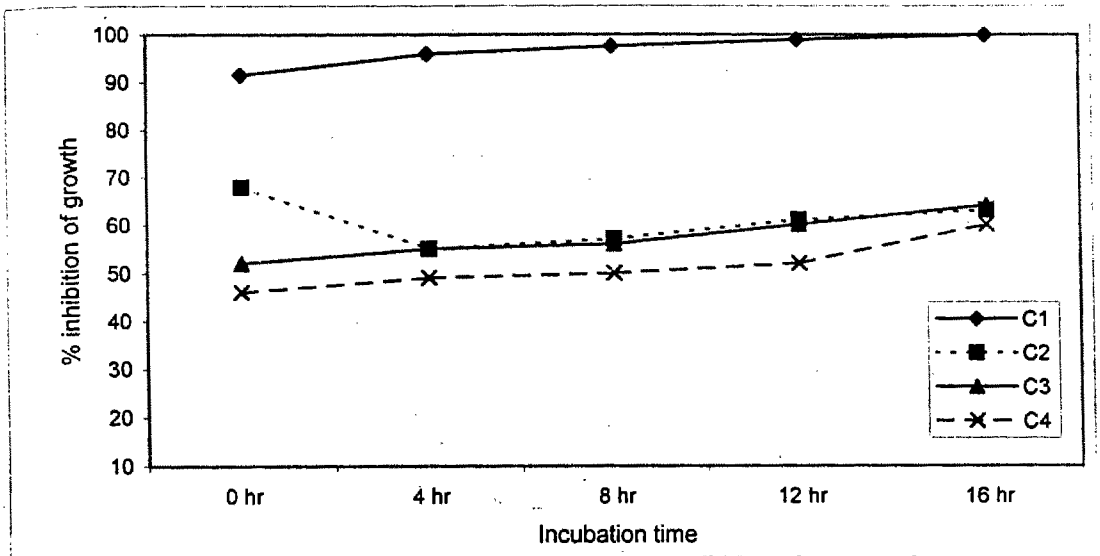

Fig 4. 28 Effect of incubation time on percent inhibition of growth of baterial culture in 0.3% bile salt concentration

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

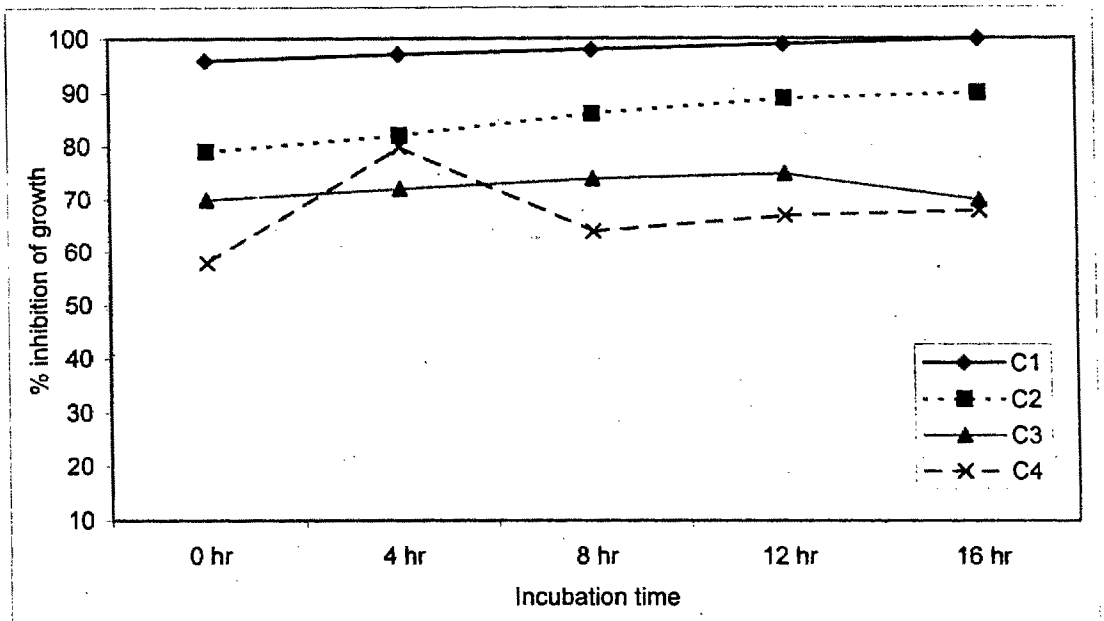

Fig. 4.29 Effect of incubation time on percent inhibition of growth of baterial culture in 0.5 % bile salt concentration

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

Amiri (2001) also reported minimum bile tolerance level of control (M_4) or conventional yoghurt cultures and that under conditions similar to the present study, *B. bifidum* led to minimum per cent growth inhibition amongst *L. casei* (M_1), *L. acidophilus* (M_2) and *B. bifidum* (M_3) when added individually to control culture in 1:1:1 ratio during 4 to 8 hrs of incubation. She also found that 0.5 per cent bile salt inhibited 61 per cent growth whereas 0.3 per cent bile salt could only inhibit 52 per cent growth of cultures. The results of present investigation are also in agreement with these findings. She also reported that the minimum and maximum inhibition of growth occurred in M_3 and M_1 cultures, respectively. The findings of present investigation are also in consonance with these observations. On addition of 0.5 per cent bile salt to lactic broth, the minimum inhibition of growth among probiotic cultures was observed in C_4 culture comprising of both probiotic bacteria namely *L. acidophilus* and *B. bifidum* with yoghurt starters, followed by C_3 culture having *B. bifidum* and C_2 culture containing *L. acidophilus* with yoghurt bacteria.

Franklin and Skoryna (1971) reported that it is likely that the ingested organism would come in contact with pH value in range of 2 to 8 as a great deal of variation in pH of stomach is observed due to presence or absence of food. It has been suggested that destruction of the microorganisms in stomach is pH dependent **(Giannella *et al.*, 1972)**. This bacteriocidal effect is very much evident at pH values below 2.5 **(Maffei and Nobrega, 1975)**. For beneficial role, an organism must be able to grow or survive in lethal

environment of gastrointestinal tract **(Sandine, 1979)**. In the present investigation, taking per cent growth inhibition as an indicator of bile tolerance, inhibition less than 65 per cent showed appreciable bile tolerance capacity of cultures. Accordingly, all the culture, except control, at pH 4-4.4 were capable to tolerate 0.3 per cent bile salt. C_4 and C_3 only could tolerate 0.5 per cent bile salt. The bile salt tolerance was found significant at 0.3 level which is normally encountered in human intestine **(Sjovall, 1959)**. The ability of *L. acidophilus* to grow at lower pH and tolerate bile salt has been reported by **Gilliand and Walker (1990)**. Furthermore, a synergistic effect of two probiotic cultures namely *L. acidoplhilus* and *B. bifidum* was met with, which led to increased bile tolerance capacities at both levels of bile salt concentration.

4.7.4 Antagonistic activity of bacterial cultures towards human pathogens

The antagonistic activity of starter cultures in different combinations (C_1-C_4) towards human pathogens was determined by well-array technique. The inhibition of three human pathogens namely *Escherichia* coli, *Staphylococcus aureus* and *Salmonella havana* was studied using the cell free filtrate of starters. After incubation for 48 h at 37±1°C, the clear zones around wells were measured by vernier calipers in mm. The results obtained are given in Table 4.35. From the data, it may be seen that the largest inhibitory zones were obtained when cell free filtrate of C_3 culture was added to the well, followed by C_4, C_2 and C_1 in case of E. *coli* and *S. aureus*. However, in case of

Table 4.35 Antagonistic activity of yoghurt-cheese cultures towards some human pathogens

Bacterial culture$ filtrate (M)	Inhibitory zones diameter in millimeter			
	Target human pathogen (S)			
	Escherichia coli	*Staphylococcus aureus*	*Salmonella havana*	Mean
C_1	14.10	12.31	13.05	13.15
C_2	14.90	15.27	12.34	14.17
C_3	17.23	17.86	14.25	16.45
C_4	16.21	16.01	13.02	15.08
Mean	15.61	15.36	13.16	14.71

Sources of variation	F value	SEM	CD (P≤0.01)
M	**	0.050	0.217
S	**	0.043	0.189
M x S	**	0.087	0.379

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Incubation temperature: 37±1^{0}C

Growth medium: Nutrient agar

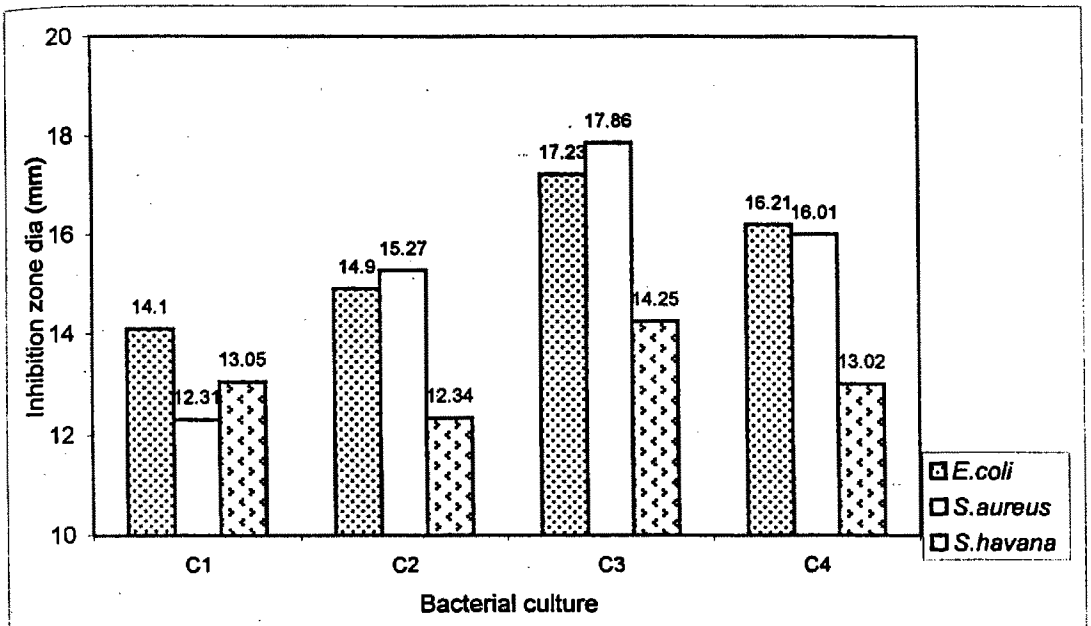

Fig. 4.30 Effect of cultures on inhibition zone diameter of pathogens

C1= *L. bulgaricus+ S.thermophilus* (1:1)

C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)

C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)

C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

S. havana, the largest zone was obtained by C_3 culture filtrate followed C_4, C_1 and C_2 (Fig. 4.30). The results indicate that *B. bifidum* has higher antagonistic activity in association with conventional yoghurt cultures against human pathogens as compared to that of *L. acidophilus*. The differences with respect to the type of culture and type of pathogens were statistically significant ($P \leq 0.01$).

Amiri (2001) has reported that among the four culture filtrates namely M_1 (yoghurt culture + *L. casei*, 1:1:1), M_2 (yoghurt cultures + *L. acidophilus*, 1:1:1), M_3 (yoghurt cultures + *B. bifidum*, 1:1:1) and M_4 (yoghurt culture), the maximum and minimum antagonistic acidity was shown by M_3 and M_4 filtrate, respectively against all three pathogens, namely *E. coli, S. auresus* and *S. typhimurium*. *L. bulgaricus* and *S. thermophilus* are reported to produce metabolites, inhibitory to pathogens, the former organism being considerably more effective against the later. Elaboration of bacteriocin or bacteriocin like compounds such as bifidin by *B. bifidum* proved to be inhibitory towards undesirable microorganism of public health **(Sarkar and Mishra, 1998)**. Inhibition of coliforms, *Bacillus cereus, Shigella dysenteriae* and *Salmonella typhimurium* by antimicrobials elaborated by mixed cultures of yoghurt organism, *B. bifidum* and *P. shermanii* have been documented by same authors. It has been reported that the antagonistic activity of *L. acidophilus* and *L. casei* may be attributed to both acid development and elaboration of antimicrobial substances **(Vervaech *et al.*, 1990; Lortie *et al.*, 1992; Vignolo *et al.*, 1993)**.

Josef *et al.* (1998) reported that the inhibition caused by some strains of the *L. acidophilus* on some strains of *S. thermophilus, L. bulgaricus* and *Bifidobacteria*. They suggested that inhibition caused could have been due to hydrogen peroxide, organic acids or some bacteriocin like inhibitory substance. This might have been the cause of lesser inhibitory effect of C_4 filtrate with regards to C_3 filtrate, towards the pathogens.

4.8 Storage study of synbiotic yoghurt-cheese

The synbiotic yoghurt-cheese samples were prepared from buffalo milk containing 0.5 per cent fat and pre-optimized levels of prebiotic ingredients and enricher (honey). The fermentation of milk was carried out using four different sets of cultures with or without probiotic bacteria to prepare yoghurt, which was then centrifuged at 9000 rpm for 15 min in order to obtain yoghurt-cheese. The cheese samples so obtained were stored at refrigeration temperature (5±1°C) and the samples were analysed for physico-chemical, microbiological and sensory characteristics at an interval of 7 days upto 28 days.

4.8.1 Physico-chemical changes during storage

4.8.1.1 pH

The yoghurt-cheese samples prepared by using different cultures had significantly ($P \leq 0.01$) different pH values. The pH values of all samples were found to decrease significantly ($P \leq 0.01$) during storage under refrigerated conditions continuously for 4 weeks. However, the addition of prebiotic

ingredients and/or honey to standardized milk had non-significant effect on the pH of yoghurt-cheese (Table 4.36). The lowest pH was obtained in cheese samples prepared by using C_4 culture followed by C_2, C_1 and C_3 (Fig. 4.31). It was found that the pH of cheese prepared by using C_3 and C_1 cultures always remained higher than those prepared by C_2 and C_4 cultures. After 21 days, reduction in pH in case of C_2 culture was greater than C_4 culture. The pH of all samples decreased at much faster rate during first 7 days of storage and thereafter a gradual and steady decrease in pH was observed.

Amiri (2001) also found that the type of probiotic cultures and their interaction had significant effect on pH of yoghurt samples. The pH of yoghurt samples prepared by using traditional yoghurt starter with or without probiotic bacteria decreased during storage. She reported that the pH of all samples decreased at much faster rate during first 11 days of storage.

Contrary to the findings of present investigation, she reported that the minimum pH was found for control sample and addition of *L. casei, L. acidophilus* or *B. bifidum* to yoghurt culture individually, exhibited more pH in comparison to control sample. But similar to the findings in current study, she also reported that *L. acidophilus* addition to yoghurt culture exhibited less pH as compared to *B. bifidum.*

Farooq and Haque (1992) have reported that the pH decreased from 4.25 to 3.84 during 14 days of storage. **Keating and White (1990)** also have reported that pH decreased upto 28 days and then it increased during 42 days of

Table 4.36 Effect of bacterial culture, prebiotic ingredients/enricher and storage period on pH of yoghurt-cheese

Bacterial culture$ (M)	Prebiotic Ingredients (S)	pH of yoghurt-cheese during storage					Mean
		Storage period in days (SS)					
		0	7	14	21	28	
C_1	Control	4.38	4.14	4.02	3.90	3.81	4.04
	Raftiline	4.40	4.15	4.03	3.91	3.82	4.04
	Raftilose	4.40	4.15	4.02	3.91	3.81	4.05
	Oat fiber	4.42	4.14	4.03	3.91	3.81	4.05
	Honey#	4.39	4.15	4.02	3.92	3.82	4.05
	Raftiline +Oat	4.40	4.15	4.02	3.92	3.82	4.06
	Raftilose+ Oat	4.38	4.14	4.02	3.91	3.82	4.05
	Honey +Oat	4.40	4.14	4.02	3.92	3.78	4.05
Mean	-	4.40	4.14	4.02	3.91	3.82	-
C_2	Control	4.33	4.10	3.97	3.86	3.76	4.03
	Raftiline	4.31	4.12	3.97	3.84	3.77	4.03
	Raftilose	4.32	4.11	3.97	3.85	3.76	4.04
	Oat fiber	4.33	4.12	3.98	3.86	3.77	4.03
	Honey	4.32	4.10	3.97	3.85	3.76	4.03
	Raftiline +Oat	4.33	4.10	3.97	3.86	3.77	4.03
	Raftilose+ Oat	4.33	4.10	3.96	3.86	3.76	4.04
	Honey +Oat	4.33	4.12	3.96	3.85	3.77	-
Mean	-	4.33	4.11	3.97	3.85	3.72	
C_3	Control	4.47	4.21	4.09	3.97	3.89	4.13
	Raftiline	4.45	4.23	4.10	3.96	3.90	4.14
	Raftilose	4.46	4.22	4.09	3.97	3.90	4.13
	Oat fiber	4.45	4.22	4.10	3.96	3.89	4.14
	Honey	4.47	4.22	4.09	3.97	3.91	4.13
	Raftiline +Oat	4.45	4.23	4.11	3.96	3.90	4.14
	Raftilose+ Oat	4.45	4.22	4.10	3.97	3.91	4.14
	Honey +Oat	4.47	4.22	4.11	3.97	3.89	4.13

Mean	-	4.46	4.22	4.10	3.97	3.90	4.14
	Control	4.30	4.07	3.94	3.83	3.75	3.98
	Raftiline	4.31	4.06	3.94	3.84	3.76	3.98
	Raftilose	4.29	4.07	3.95	3.83	3.75	3.97
C_4	Oat fiber	4.31	4.07	3.95	3.83	3.76	3.98
	Honey	4.30	4.07	3.95	3.85	3.75	3.98
	Raftiline +Oat	4.30	4.06	3.95	3.84	3.75	3.98
	Raftilose+ Oat	4.30	4.06	3.94	3.84	3.75	3.97
	Honey +Oat	4.29	4.07	3.94	3.83	3.74	3.98
Mean	-	4.30	4.06	3.95	3.84	3.75	-

Sources of variation	F value	SEM	CD (P<0.05)
M	**	0.000	0.001
S	ns	0.000	0.002
SS	**	0.000	0.002
M x S	**	0.001	0.002
S x SS	**	0.002	0.003
M x SS	**	0.001	0.005
M x S x SS	**	0.003	0.011

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

Level of ingredients: Raftiline (4%), Raftilose (4%), Oat fiber (1%), Honey (3%), Raftiline + Oat fiber (4+0.5%), Raftilose + Oat fiber (4+0.5%) and Honey + Oat fiber (3+0.5%)

Storage temperature: 5±1 ^{0}C

storage. Their studies thus confirm our finding of decrease in pH during 28 days of storage in all cheese samples.

2.8.1.2 Titratable acidity

The titratable acidity and pH of yoghurt-cheese during storage had inverse relationships. The cheese samples prepared by using different set of cultures had significantly ($P \leq 0.01$) different titratable acidity throughout the storage. The storage period also caused a significant ($P \leq 0.01$) increase in titratable acidity of cheese samples. Similar to pH, the acidity of yoghurt-cheese also remained unaffected by the addition of optimized levels of prebiotic ingredients and enricher (Table 4.37). Just after preparation of cheese, its acidity level ranged between 1.01-1.19 per cent as lactic acid depending upon the type of culture used for its preparation. At the end of storage after 28 days, the titratable acidity of different samples ranged between 1.51 and 1.67 per cent as lactic acid. Throughout the storage period, the highest acidity values were obtained in yoghurt-cheese samples prepared by C_4 cultures, followed by C_2, C_1 and C_3 (Fig 4.32). This indicates that *L. acidophilus* produced more acidity than *B. bifidum* singly or in combination. Slightly higher rate of acidity was observed during first week of storage of cheese samples, which was followed by a gradual increase in all remaining stages of storage.

Samona *et al.* (1996) have reported that the acid concentration remained quite stable in samples having *B. bifidum* along with yoghurt-culture, whereas,

Table 4.37 Effect of bacterial culture, prebiotic ingredients/enricher and storage period on titratable acidity of yoghurt-cheese

Bacterial culture$(M)	Prebiotic Ingredient (S)	TA (% lactic acid) of yoghurt-cheese during storage					Mean
		Storage period in days (SS)					
		0	7	14	21	28	
	Control	1.11	1.34	1.45	1.53	1.61	1.41
	Raftiline	1.12	1.35	1.44	1.54	1.60	1.41
	Raftilose	1.11	1.34	1.45	1.55	1.60	1.42
C_1	Oat fiber	1.12	1.35	1.45	1.53	1.60	1.41
	Honey#	1.12	1.34	1.44	1.54	1.61	1.42
	Raftiline +Oat	1.13	1.35	1.45	1.54	1.62	1.42
	Raftilose+ Oat	1.13	1.35	1.45	1.55	1.61	1.41
	Honey +Oat	1.12	1.34	1.46	1.54	1.63	1.41
Mean	-	1.12	1.34	1.45	1.54	1.61	-
	Control	1.16	1.36	1.47	1.58	1.65	1.44
	Raftiline	1.17	1.35	1.47	1.56	1.66	1.43
	Raftilose	1.16	1.36	1.48	1.56	1.64	1.43
C_2	Oat fiber	1.17	1.36	1.48	1.57	1.64	1.44
	Honey	1.17	1.35	1.47	1.57	1.65	1.44
	Raftiline +Oat	1.18	1.35	1.48	1.57	1.65	1.44
	Raftilose+ Oat	1.16	1.35	1.47	1.57	1.64	1.43
	Honey +Oat	1.16	1.36	1.48	1.56	1.64	1.44
Mean	-	1.17	1.35	1.47	1.57	1.64	-
	Control	1.02	1.26	1.37	1.45	1.52	1.32
	Raftiline	1.01	1.25	1.36	1.45	1.51	1.31
	Raftilose	1.02	1.26	1.38	1.46	1.52	1.32
C_3	Oat fiber	1.02	1.26	1.38	1.44	1.51	1.31
	Honey	1.02	1.27	1.36	1.44	1.51	1.32
	Raftiline +Oat	1.03	1.27	1.38	1.44	1.52	1.32
	Raftilose+ Oat	1.01	1.25	1.37	1.45	1.52	1.31
	Honey +Oat	1.03	1.26	1.36	1.46	1.52	1.32

Mean	-	1.02	1.26	1.37	1.45	1.52	-
	Control	1.19	1.40	1.51	1.59	1.66	1.47
	Raftiline	1.20	1.41	1.52	1.59	1.67	1.48
	Raftilose	1.19	1.42	1.52	1.60	1.67	1.47
C_4	Oat fiber	1.20	1.41	1.51	1.61	1.67	1.48
	Honey	1.18	1.42	1.51	1.61	1.66	1.47
	Raftiline +Oat	1.19	1.41	1.51	1.60	1.66	1.48
	Raftilose+ Oat	1.19	1.40	1.52	1.60	1.67	1.47
	Honey +Oat	1.19	1.41	1.51	1.60	1.66	1.47
Mean	-	1.19	1.41	1.51	1.60	1.66	-

Sources of variation	F value	SEM	CD (P<0.05)
M	**	0.000	0.001
S	ns	0.001	-
SS	**	0.001	0.002
M x S	**	0.002	0.005
S x SS	**	0.001	0.003
M x SS	**	0.002	0.005
M x S x SS	**	0.004	0.011

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

Level of ingredients: Raftiline (4%), Raftilose (4%), Oat fiber (1%), Honey (3%), Raftiline + Oat fiber (4+0.5%), Raftilose + Oat fiber (4+0.5%) and Honey + Oat fiber (3+0.5%)

Storage temperature: 5±1 ^{0}C

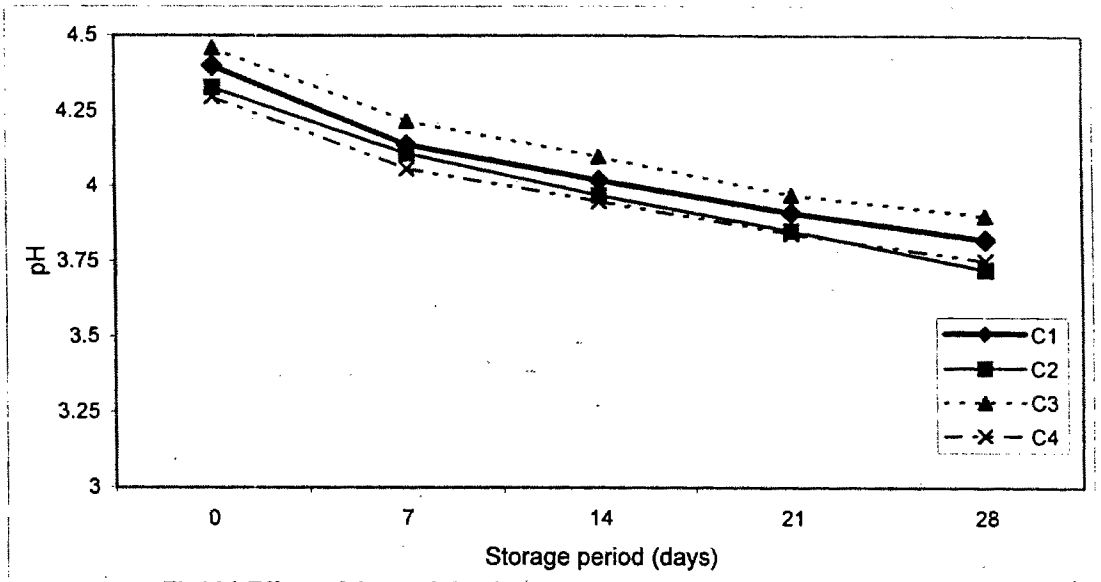

Fig4.31 Effect of bacterial culture and storage period on pH of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

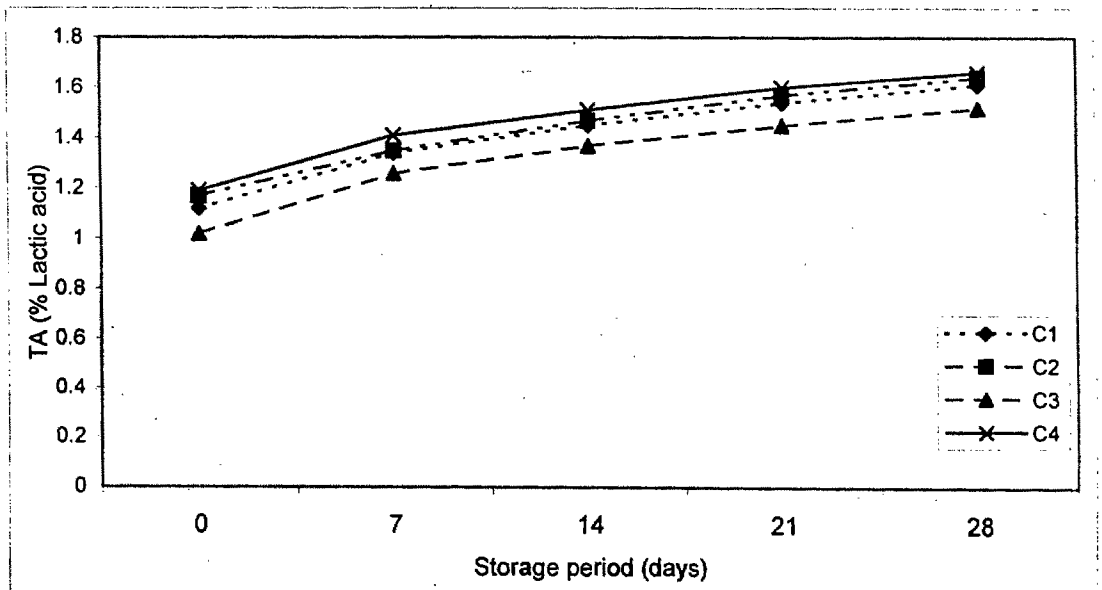

Fig.4.32 Effect of bacterial culture and storage period on Titratable acidity of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

Sarkar and Mishra (1998) have reported that titratable acidity of yoghurt sample having *B. bifidum*, yoghurt culture and *Freudenreichii shermanii*, increased gradually during 7 days of storage at 8°C. The later study confirms our finding of increase in acidity in samples prepared by C_3 cultures combination containing *B. bifidum.*

Amiri (2001) has also reported that the titratable acidity was minimum at the beginning of storage and it increased till three quarters of storage period, thus confirming our findings. But unlike in present investigation, the titratable acidity decreased thereafter till end of storage. In contrast to our findings, she found that yoghurt prepared using control culture yielded maximum acidity (1.3%) and yoghurt prepared by incorporation of *L. acidophilus* in control culture yielded minimum acidity (0.975%). She reported that addition of either of the probiotic culture namely *L. casei, L. acidophilus* or *B. bifidum* in control culture led to decrease in acidity of yoghurt whereas reverse observations were met with during present investigation. It was revealed in the present study that C_4 culture led to maximum acidity development that might be attributed to synergistic activity of all four bacterial present in it. The studies of **Sarkar and Mishra (1998), Vervaeck *et al.* (1990)** and **Vingolo *et al.* (1993)** invariably reported that inhibitive power of cultures towards pathogens was a joint result of all bacteria, indicating synergism between them.

4.8.1.3 Soluble nitrogen

The per cent soluble nitrogen content of yoghurt-cheese samples at zero day of storage ranged between 0.126 and 0.169 per cent depending upon the culture used for the yoghurt preparation. The variations in soluble nitrogen content of cheese samples prepared by different set of cultures were significant ($P \leq 0.01$). The soluble nitrogen content of cheeses also differed significantly ($P \leq 0.01$) by prebiotic ingredients/enricher addition to standardized milk and the period of storage (Table 4.38). After 28 days of storage, the soluble nitrogen content of cheese samples ranged between 0.15-0.23 per cent depending upon the type of culture and prebiotic ingredient/honey used for its preparation. The maximum soluble nitrogen was at the end of storage period obtained in control samples, followed by samples prepared using C_4, C_3 and C_2 cultures (Fig. 4.33). These variations in soluble nitrogen content of cheese samples prepared by using different bacterial cultures could be attributed to differences in proteolytic activity of cultures. Among the prebiotic ingredients and honey, the combinations of oat fiber with raftiline, raftilose and honey gave higher soluble nitrogen content in cheese samples. The minimum per cent soluble nitrogen was obtained in cheese prepared without addition of neither of prebiotic ingredient nor honey.

It has been reported by **Rajagopal and Sandine (1990)** that lactobacilli were highly proteolytic (61-144.6 µg of tyrosine/ml of milk). *S. thermophilus* was also less proteolytic (2.4-14.8 µg of tyrosine/ml of milk) and *S.*

Table 4.38 Effect of bacterial culture, prebiotic ingredient/enricher and storage period on soluble nitrogen content of yoghurt-cheese

Bacterial culture$(M)	Prebiotic Ingredient (S)	% Soluble nitrogen					Mean
		Storage period in days (SS)					
		0	7	14	21	28	
C1	Control	0.152	0.183	0.202	0.226	0.215	0.196
	Raftiline	0.166	0.189	0.211	0.234	0.221	0.204
	Raftilose	0.167	0.189	0.212	0.234	0.221	0.204
	Oat fiber	0.169	0.169	0.211	0.234	0.222	0.199
	Honey#	0.152	0.182	0.202	0.227	0.216	0.196
	Raftiline +Oat	0.168	0.192	0.214	0.239	0.230	0.209
	Raftilose+ Oat	0.169	0.191	0.214	0.238	0.231	0.209
	Honey +Oat	0.170	0.191	0.213	0.238	0.230	0.208
Mean	-	0.164	0.185	0.210	0.234	0.223	-
C2	Control	0.125	0.145	0.170	0.167	0.154	0.153
	Raftiline	0.135	0.155	0.834	0.173	0.164	0.162
	Raftilose	0.134	0.154	0.182	0.172	0.163	0.161
	Oat fiber	0.139	0.158	0.185	0.177	0.170	0.166
	Honey	0.126	0.146	0.171	0.167	0.154	0.153
	Raftiline +Oat	0.138	0.154	0.183	0.176	0.169	0.164
	Raftilose+ Oat	0.139	0.154	0.183	0.177	0.168	0.165
	Honey +Oat	0.137	0.154	0.184	0.176	0.168	0.164
Mean	-	0.134	0.152	0.181	0.173	0.164	-
C3	Control	0.136	0.154	0.181	0.172	0.165	0.162
	Raftiline	0.143	0.163	0.189	0.182	0.174	0.170
	Raftilose	0.143	0.164	0.187	0.181	0.173	0.169
	Oat fiber	0.150	0.169	0.194	0.190	0.181	0.177
	Honey	0.136	0.155	0.182	0.172	0.164	0.162
	Raftiline +Oat	0.150	0.169	0.194	0.191	0.178	0.176
	Raftilose+ Oat	0.150	0.170	0.194	0.190	0.178	0.175

	Honey +Oat	0.150	0.170	0.194	0.191	0.178	0.176
Mean	-	0.144	0.164	0.189	0.184	0.174	-
	Control	0.141	0.162	0.198	0.175	0.164	0.168
	Raftiline	0.147	0.168	0.205	0.182	0.172	0.175
	Raftilose	0.147	0.167	0.204	0.182	0.172	0.174
C4	Oat fiber	0.154	0.173	0.210	0.188	0.177	0.181
	Honey	0.140	0.163	0.196	0.174	0.164	0.167
	Raftiline +Oat	0.152	0.170	0.209	0.187	0.175	0.179
	Raftilose+ Oat	0.151	0.170	0.208	0.186	0.174	0.178
	Honey +Oat	0.151	0.170	0.208	0.186	0.175	0.178
Mean	-	0.148	0.168	0.205	0.182	0.172	-

Sources of variation	F value	SEM	CD (P<0.05)
M	**	0.000	0.001
S	**	0.000	0.001
SS	**	0.000	0.001
M x S	**	0.001	0.003
S x SS	ns	0.002	-
M x S	**	0.001	0.002
M x S x SS	ns	0.002	-

S C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher

Level of ingredients: Raftiline (4%), Raftilose (4%), Oat fiber (1%), Honey (3%), Raftiline + Oat fiber (4+0.5%), Raftilose + Oat fiber (4+0.5%) and Honey + Oat fiber (3+0.5%)

thermophilus was less proteolytic (2.4-14.8 µg of tyrosine/ml of milk). Mixed culture of both liberated more tyrosine (92.6-419.9 µg/ml) than the sum of the individual cultures. This confirms our findings as C_1 culture having both *L. bulgaricus* and *S. thermophilus* yielded maximum soluble nitrogen content. **Sarkar and Mishra (1998)** reported that proteolytic activity of yoghurt having yoghurt culture, *B. bifidum and Freudenreichii Shesmanii* increased and reached to their peak value after 3 days and then decreased after 7 days of storage. The present study revealed that the per cent soluble nitrogen content increased continuously till 21 days of storage and decreased thereafter. **Amiri (2001)** also reported met with the same observation that soluble nitrogen content of yoghurt samples increased continuously till 22 days of storage and started decreasing thereafter when probiotic bacteria were used along with control culture. Whereas in control yoghurt culture, the soluble nitrogen content increased till end of storage. She also reported that *B. bifidum* and *L. acidophilus* decreased the proteolytic activity of yoghurt samples and that *L. acidophilus* decreased the proteolytic activity at higher rate than that of *B. bifidum*. This confirms our findings of effect of cultures on soluble nitrogen content of yoghurt-cheese samples.

4.8.2 Microbiological analysis

The cheese samples prepared by using different bacterial cultures were analysed for total viable count, yeast and mold count, proteolytic count and coliform count after every 7 days upto 28 days. As there was no variation in

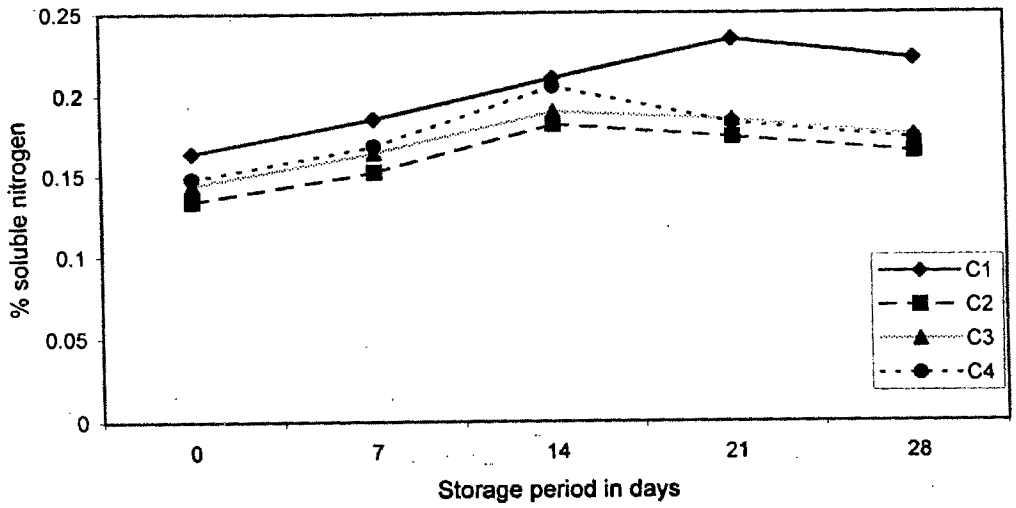

Fig 4.33 Effect of bacterial culture and storage period on soluble nitogen content of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

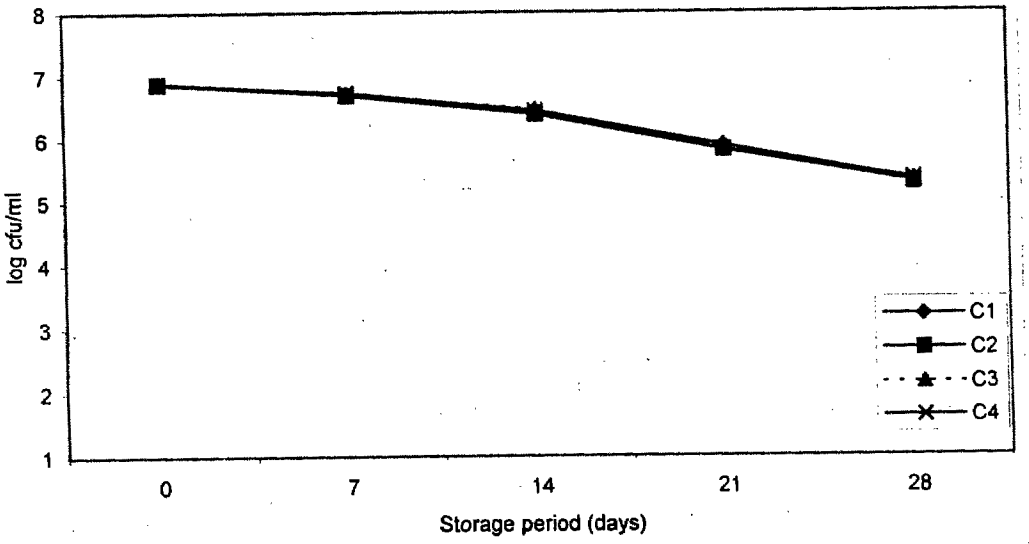

Figure 4.34 Effect of bacterial cultures and storage period on total viable count of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

the samples prepared after adding prebiotic ingredients and enricher only storage time and type of culture were taken as variables.

4.8.2.1 Total viable count

The total viable count of the cheese samples decreased significantly ($P \leq 0.01$) during storage. However, there was a non-significant variation among the total viable counts of yoghurt-cheese samples prepared by using different cultures (Table 4.39). It may be seen from Fig. 4.34 that among the different bacterial cultures, maximum viable count was obtained in C_1 culture followed by C_3, C_2 and C_4 cultures. Viable counts of all cheese samples prepared by using different starter culture were nearly same upto 21 days of storage and only slight decrease in viable count was observed thereafter, in case of yoghurt-cheese prepared by C_3 culture as compared to other cultures. The total viable counts in this study were in acceptable range till 14 days of storage. **Joseph *et al.* (1998)** have reported that the required minimum level of probiotic bacteria is claimed to be 10^6 cfu/g of product, whereas, **Tanaka *et al.*, (1982)** and **Kim (1988)** have reported that viable population of 10^8-10^9 cell/ml of the product is required for successful implementation in intestine. **Joseph *et al* (1998)** have indicated some antagonistic property between the probiotic cultures and yoghurt bacteria.

Amiri (2001) had also observed that total viable counts in yoghurt significantly decreased during storage period of 4 weeks. **Yadav *et al.* (1994)** have also reported that viable counts gradually decreased during storage period.

Table 4.39 Changes in total viable count of yoghurt-cheese during storage

Bacterial culture$ (M)	Total viable count (cfu/ml)				
	Storage period in days (S)				
	0	7	14	21	28
C_1	7.93×10^6	5.34×10^6	2.82×10^6	7.76×10^5	2.13×10^5
C_2	7.77×10^6	5.02×10^6	2.43×10^6	6.58×10^5	2.01×10^5
C_3	7.81×10^6	5.24×10^6	2.99×10^6	7.14×10^5	1.91×10^5
C_4	7.62×10^6	5.47×10^6	2.79×10^6	6.66×10^5	2.24×10^5

Source of variation	F value	SEM	CD (P<0.01)
M	ns	0.105	-
S	**	0.118	0.047
M x S	ns	0.024	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Storage temperature: 5 ± 1^0 C

Medium used: Lactic agar

Incubation temperature and time: 37^0 C for 24-48 h

Thus our findings are in agreement with above studies, whereas **Keating and White (1990)** have reported that bacterial counts decreased non-significantly during the first 14 days of storage and thereafter decreased significantly till the end of 42 days of storage of yoghurt-cheese.

4.8.2.2 Yeast and mold counts

Yeast and mold count in all the cheese samples prepared by four set of starter cultures could not be detected upto 14 days of storage and thereafter, the yeast and mold count increased significantly ($P \leq 0.01$) till end of storage (Table 4.40). The cheese samples prepared by using different set of cultures also differed significantly among themselves with respect to yeast and mold count. The maximum final yeast and mold count was detected in case of cheese prepared by C_3 culture followed by C_4, C_1 and C_2 (Fig. 4.35).

Contamination by yeasts and molds is one of the limiting factor for the stability and commercial value of yoghurt **(Suriyarachchi and Fleet, 1981; Deak, 1991). Amiri (2001)** has also reported that type of culture and storage period significantly effected yeast and mold count of probiotic yoghurt samples. She also did not observe yeast and molds apparently till 16 days of storage, which increased thereafter till 28 days of storage. **Salji *et al.* (1987)** have also reported that yeast and mold counts increased from 1 to 22 cfu/ml after 2 weeks of storage. Thus, the results of present investigation are in consonance with above findings. The yeast and mold count in the present

Table 4.40 Changes in yeast and mold count of yoghurt-cheese during storage

Bacterial culture$ (M)	Yeast and mold count (cfu/ml)				
	Storage period in days (S)				
	0	7	14	21	28
C_1	-	-	-	8.0	16
C_2	-	-	-	6.5	10
C_3	-	-	-	5.5	21
C_4	-	-	-	7.5	19

Sources of variation	F value	SEM	CD (P≤0.01)
M	ns	0.235	-
S	**	0.026	0.105
M x S	ns	0.052	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Storage temperature: 5 ± 1^0 C

Medium used: Potato dextrose agar

Incubation temperature and time: 22 ± 1^0 C for 2-4 days

study were more in samples prepared by C_4, C_2 and C_1 cultures, which might have been due to lower pH of these samples.

4.8.2.3 Proteolytic counts

The proteolytic counts appeared only after 7 days in all the cheese samples and thereafter increased significantly ($P \leq 0.01$) during the rest of storage period. The proteolytic counts of yoghurt-cheese samples prepared by different bacterial cultures were also significantly ($P \leq 0.01$) different (Table 4.41). It may be seen from Fig. 4.36 that the samples prepared by C_1 culture had the highest proteolytic counts followed by those prepared by C_2, C_4 and C_3 cultures at all stages of determination during storage. Some correlation existed between per cent soluble nitrogen content and proteolytic count in yoghurt cheese samples prepared by different bacterial cultures as it was observed that the cheese sample having higher soluble nitrogen content also harboured more proteolytic bacteria in it. **Salji *et al.* (1987)** have also reported that the proteolytic count of yoghurt increased continuously during the storage period. It was observed in present investigation that the proteolytic counts increased continuously from 7 days to the end of storage period.

4.8.2.4 Coliform counts

The samples of cheese were also analysed for the presence of coliforms. Normally the coliforms were absent in the samples but occasionally presence

Table 4.41 Changes in proteolytic count of yoghurt-cheese during storage

Bacterial culture$ (M)	Proteolytic count (cfu/ml)				
	Storage period in days (S)				
	0	7	14	21	28
C_1	-	4.50	43.00	138.50	218.50
C_2	-	6.00	25.00	108.00	170.50
C_3	-	4.50	17.00	118.50	176.00
C_4	-	7.30	31.00	127.50	200.50

Sources of variation	F value	SEM	CD (P≤0.01)
M	**	0.016	0.066
S	**	0.018	0.074
M x S	ns	0.037	-

$ C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Storage temperature: 5 ± 1^0 C

Medium used: Skim milk agar

Incubation temperature and time: 37^0 C for 24-48 h

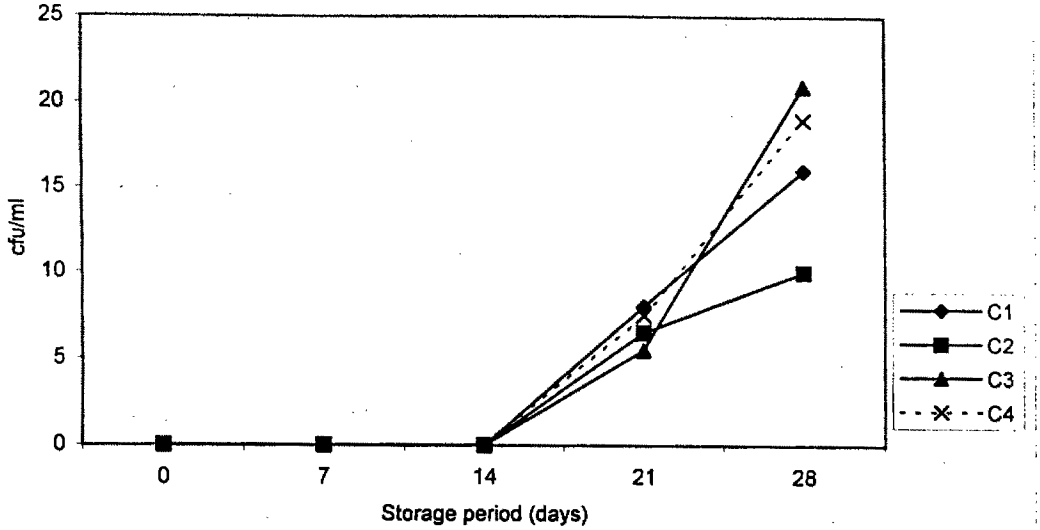

Figure 4.35 Effect of bacterial cultures and storage period on yeast and mold count of cultures

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

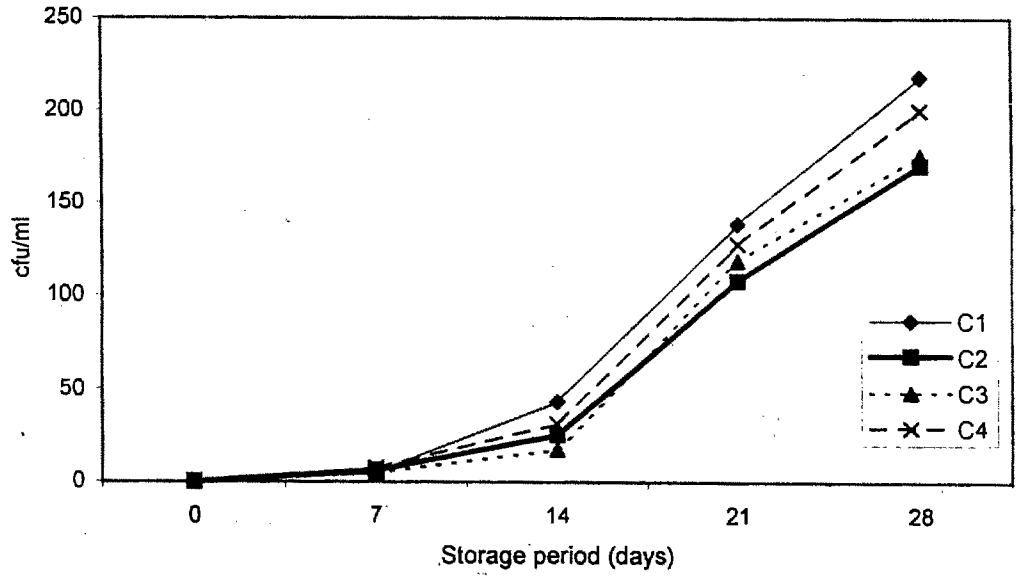

Figure 4.36 Effect of intercation of bacterial cultures and storage period on proteolytic count of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1)
C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)
C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)
C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

of 1-2 colonies was detected which might have entered the cheese samples as contaminants.

4.8.3 Changes in sensory quality

The cheese samples prepared after adding various prebiotic ingredients and /or enricher using different bacterial cultures were subjected to sensory evaluation with respect to colour, flavour, texture and overall acceptability on 9-Point hedonic scale.

4.8.3.1 Colour

No effect was observed on colour of yoghurt-cheese samples prepared by using different cultures and therefore, this parameter was deleted while analyzing the data statistically. The colour of cheese samples prepared from standardized milk containing optimized levels of prebiotic ingredient and/or honey differed with each other significantly ($P \leq 0.01$) with respect to sensory score of colour. During storage, the sensory score for colour of all samples decreased significantly ($P \leq 0.01$) from 7 days of storage till end of storage period (Table 4.42). The mean sensory scores of all the samples obtained after 7 days of storage upto 28 days are shown in Fig 4.37. It was revealed that during first 7 days of storage, the sensory score for colour remained same and thereafter a steady decrease in scores was obtained. Among additives used, oat and honey decreased the sensory score of colour of yoghurt-cheese. **Amiri (2001)** also observed no change in colour of yoghurt samples prepared using different starter cultures.

Table 42 Effect of prebiotic ingredients/enricher and storage period on sensory scores for colour of yoghurt-cheese

Prebiotic ingredient in yoghurt-cheese (M)	Sensory score for colour				
	Storage period in days (S)				
-	0	7	14	21	28
Control	8.216	8.216	7.785	7.618	7.466
Raftiline	8.216	8.126	7.838	7.694	7.488
Raftilose	8.211	8.211	7.827	7.688	7.489
Oat fiber	7.877	7.877	7.655	7.283	7.050
Honey#	8.055	8.055	7.811	7.672	7.427
Raftiline + Oat fiber	8.172	8.172	7.816	7.622	7.472
Raftilose + Oat fiber	8.172	8.033	8.033	7.622	7.472
Honey + Oat fiber	7.977	7.977	7.638	7.422	7.250
Mean	8.110	8.095	7.800	7.578	7.389

Sources of variation	F value	SEM	CD (P≤0.01)
M	**	0.007	0.027
S	**	0.006	0.021
M x S	**	0.017	0.062

Enricher

Level of ingredients: Raftiline (4%), Raftilose (4%), Oat fiber (1%), Honey (3%), Raftiline + Oat fiber (4+0.5%), Raftilose + Oat fiber (4+0.5%) and Honey + Oat fiber (3+0.5%)

Storage temperature: 5±1 ^{0}C

4.8.3.2 Flavour

The sensory score for the flavour of cheese samples decreased significantly ($P \leq 0.01$) during storage (Table 4.43). The type of culture and prebiotic ingredient and/or honey added to standardized milk also affected the flavour of cheese significantly ($P \leq 0.01$) throughout the storage. The interactions among the type of cultures, prebiotic ingredients added and the storage period were also significant ($P \leq 0.01$). The highest sensory scores were obtained for flavour in yoghurt prepared by using C_3 culture followed by C_4, C_1 and C_2. The Fig. 4.38 shows that scores for flavour remained constant till 7 days of storage followed by a decrease during the remaining storage period in case of all cultures used in the present investigation. A constant decrease in sensory scores was probably observed throughout the storage period due to the biochemical changes brought about by the microbes present in the samples. The acidic flavour developed in samples prepared by using C_2 and C_1 cultures within 3 weeks of storage resulting in lower sensory scores for flavour. Among the additives used in preparation of yoghurt-cheese, highest scores for flavour was obtained for samples having honey followed by raftiline, raftilose and oat fiber. When ingredients were used in combination, yoghurt-cheese having honey along with oat fiber scored maximum followed by raftiline with oat fiber and raftilose with oat fiber. Thus a quite acceptable synbiotic yoghurt-cheese could be prepared after adding prebiotic fibers by using *B. bifidum* with yoghurt starters for fermentation.

Table 4.43 Effect of bacterial culture, prebiotic ingredient and storage period on sensory quality (flavour) of yoghurt-cheese

Bacterial culture$(M)	Prebiotic Ingredient (S)	Sensory score (flavour) of yoghurt-cheese during storage					Mean
		Storage period (in days)					
		0	7	14	21	28	
	Control	7.46	7.46	7.30	7.02	6.72	7.19
	Raftiline	8.02	8.02	7.55	7.14	6.82	7.51
	Raftilose	8.02	8.02	7.56	7.14	6.72	7.49
C_1	Oat fiber	7.23	7.18	7.03	6.80	6.72	6.95
	Honey#	8.07	8.07	7.73	7.43	6.99	7.66
	Raftiline +Oat	7.82	7.82	7.35	7.10	6.68	7.35
	Raftilose+ Oat	7.75	7.75	7.22	7.10	6.54	7.27
	Honey +Oat	7.88	7.88	7.40	7.20	6.95	7.46
Mean	-	7.78	7.77	7.39	7.11	6.76	-
	Control	7.28	7.28	7.02	6.93	6.35	6.97
	Raftiline	7.79	7.79	7.55	7.14	6.76	7.41
	Raftilose	7.70	7.70	7.50	7.10	6.75	7.35
C_2	Oat fiber	7.01	7.01	6.74	6.52	6.27	6.71
	Honey	7.78	7.78	7.25	7.10	6.65	7.31
	Raftiline +Oat	7.65	7.65	7.24	7.10	6.65	7.25
	Raftilose+ Oat	7.60	7.55	7.20	7.00	6.50	7.17
	Honey +Oat	7.74	7.74	7.39	7.07	6.77	7.34
Mean	-	7.56	7.56	7.23	6.99	6.58	-
	Control	7.87	7.87	7.13	6.93	6.70	7.31
	Raftiline	8.26	8.26	7.80	7.56	7.14	7.80
	Raftilose	8.16	8.16	7.68	7.42	7.02	7.69
C_3	Oat fiber	7.19	7.19	6.79	6.44	6.35	6.79
	Honey	8.10	8.10	7.75	7.45	7.15	7.71
	Raftiline +Oat	8.03	8.03	7.44	7.23	7.05	7.56
	Raftilose+ Oat	8.00	8.00	7.25	7.10	6.85	7.44

	Honey +Oat	8.10	8.10	7.73	7.38	7.10	7.68
Mean	-	8.00	8.00	7.47	7.35	6.94	-
	Control	7.72	7.72	7.27	7.01	6.65	7.27
	Raftiline	8.12	8.12	8.03	7.55	7.05	7.77
	Raftilose	8.03	8.03	7.76	7.45	7.05	7.66
C_4	Oat fiber	7.19	7.19	6.75	6.40	6.25	6.76
	Honey	8.00	8.00	7.55	7.37	7.10	7.60
	Raftiline +Oat	8.00	7.86	7.31	7.27	6.95	7.47
	Raftilose+ Oat	7.94	7.94	7.09	7.09	6.85	7.38
	Honey +Oat	8.03	8.03	7.38	7.11	6.95	7.5
Mean	-	7.92	7.86	7.39	7.15	6.85	-

Sources of variation	F value	SEM	CD (P<0.05)
M	**	0.005	0.002
S	**	0.007	0.010
SS	**	0.006	0.003
M x S	**	0.015	0.001
S x SS	**	0.011	0.010
M x S	**	0.017	0.002
M x S x SS	**	0.033	0.149

C_1 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*
C_2 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*
C_3 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Bifidobacterium bifidum*
C_4 *Lactobacillus delbrueckii sub sp. bulgaricus*: *Streptococcus salivarius* subsp. *thermophilus*: *Lactobacillus acidophilus*: *Bifidobacterium bifidum*

Enricher
Level of ingredients: Raftiline (4%), Raftilose (4%), Oat fiber (1%), Honey (3%), Raftiline + Oat fiber (4+0.5%), Raftilose + Oat fiber (4+0.5%) and Honey + Oat fiber (3+0.5%)

Storage temperature: 5±1 ^{0}C

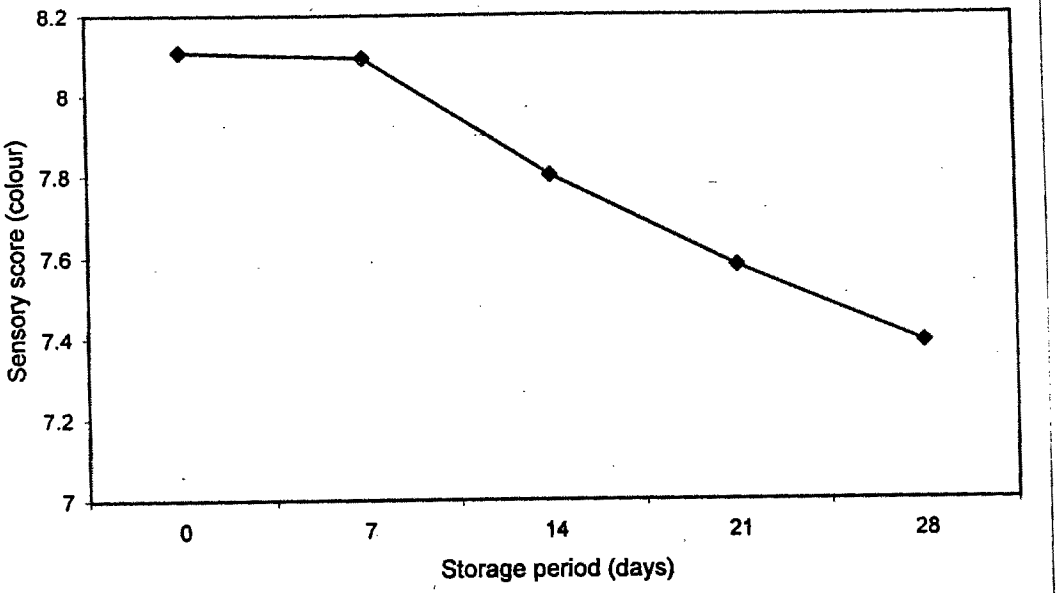

Fig. 4.37 Effect of storage period on mean sensory score for colour of yoghurt-cheese

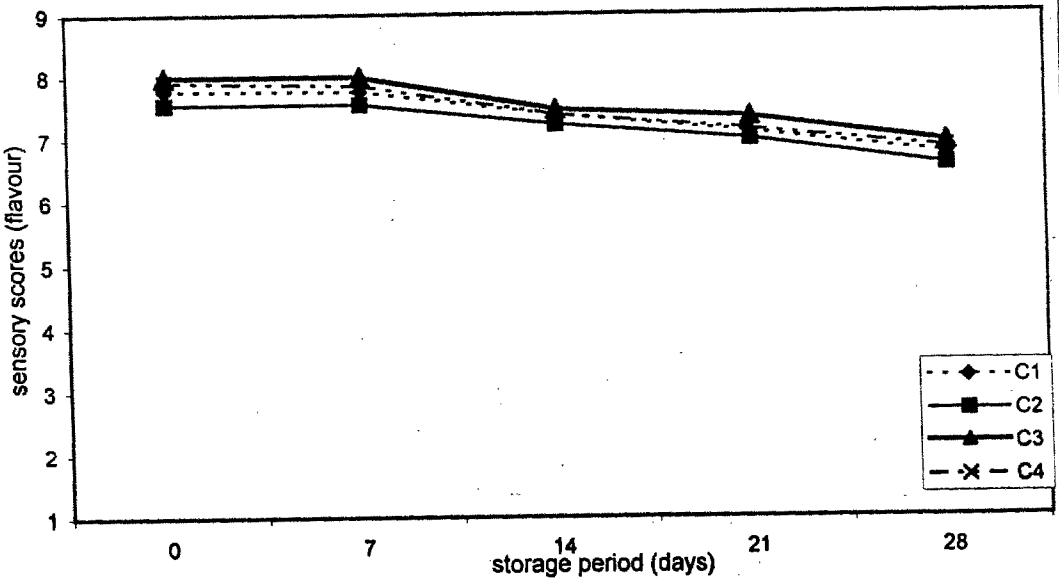

Fig. 4.38 Effect of bacterial culture and storage period on mean sensory scores for flavour of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1)

C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)

C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)

C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

Keating and White (1990) reported that there was no difference in the flavour score for the first 28 days of storage at 7°C of sucrose and sorbitol sweetened yoghurt samples and thereafter decreased significantly. But **Amiri (2001)** observed that the flavour score of yoghurt samples remained same upto 11 days of storage of 7±1°C and thereafter a progressive decline during the remaining storage period. She reported that samples prepared by using *L. casei* or *B. bifidum* with yoghurt starters were rated highest for sensory scores followed by samples prepared using *L. acidophilus* with yoghurt culture and yoghurt culture alone, respectively. Thus the current findings are in consonance with her observations.

4.8.3.3 Texture

The type of starter culture had no effect on the textural characteristics of yoghurt-cheese. The data presented in Table 4.44 show the effect of addition of prebiotic ingredients and enricher to the standardized milk and storage period on the sensory scores for texture of yoghurt-cheese. The sensory scores for texture of yoghurt-cheese were significantly ($P \leq 0.01$) affected by the type of additive in milk and storage time. The scores for texture of cheese decreased throughout the storage of 28 days. The yoghurt-cheese samples prepared after addition of raftiline, raftilose and honey and their combinations with oat fiber to standardized milk had, significantly ($P \leq 0.01$) higher sensory scores for texture as compared to those of control throughout the storage study. It may be seen from Fig. 4.39 that the sensory scores for texture of yoghurt-cheese

Table 4.44 Effect of prebiotic ingredients and storage period on sensory quality (texture) of different yoghurt-cheese samples

Prebiotic ingredient in yoghurt-cheese (M)	Sensory score for texture				
	Storage period in days (S)				
-	0	7	14	21	28
Control	7.826	7572	7.400	6.938	6.700
Raftiline	8.161	7.744	7.416	7.083	6.833
Raftilose	8.122	7.711	7.416	7.139	6.889
Oat	7.777	7.405	7.166	6.972	6.705
Honey#	8.155	7.582	7.166	6.861	6.611
Raftiline + Oat	8.105	7.805	7.166	6.833	6.761
Raftilose + Oat	8.088	7.777	7.166	6.8333	6.716
Honey + Oat	7.889	7.644	7.216	6.972	6.611
Mean	8.015	7.655	7.264	6.954	6.728

Sources of variation	F value	SEM	CD (P≤0.01)
M	**	0.020	0.073
S	**	0.058	0.058
M x S	**	0.044	0.164

Enricher

Level of ingredients: Raftiline (4%), Raftilose (4%), Oat fiber (1%), Honey (3%), Raftiline + Oat fiber (4+0.5%), Raftilose + Oat fiber (4+0.5%) and Honey + Oat fiber (3+0.5%)

Storage temperature: 5±1 ^{0}C

decreased continuously during storage but even after 28 days of storage, the sensory scores remained within acceptable limits.

Amiri (2001) has also reported that the body and texture scores for all samples prepared using different cultures decreased during storage period. Contrary to the findings of present investigation, **Keating and White (1990)** have reported that the body and texture scores of sucrose replaced yoghurt significantly increased during prolonged storage (28 days) and the body and texture score of sucrose plain yoghurt progressively improved.

4.8.3.4 Overall acceptability

The panelists were asked to access the overall acceptability of yoghurt-cheese considering its colour, flavour, texture and other sensory attributes. The overall acceptability of yoghurt-cheese was affected by the type of culture, prebiotic ingredients and enricher added to the standardized milk as well as the storage period. The sensory scores for overall acceptability remained constant till 7 days of storage and thereafter a decrease was observed till end of storage (Table 4.45). Among the prebiotic ingredients, raftiline, raftilose and honey singly and on combination with oat fiber improved the sensory score of overall acceptability significantly ($P \leq 0.01$). However, use of oat fiber for cheese preparation resulted in a significant ($P \leq 0.01$) decrease of sensory score of overall acceptability. The Fig. 40 shows the effect of type of culture used for preparation of yoghurt cheese on the overall sensory quality of the product during storage. It was revealed that C_3 culture gave yoghurt cheese with

Table 4.45 Effect of bacterial culture, prebiotic ingredient and storage period on sensory quality (OAA) of yoghurt-cheese

Bacterial culture$(M)	Prebiotic Ingredient (S)	Sensory score (OAA) of yoghurt-cheese during storage					Mean
		Storage period in days (SS)					
		0	7	14	21	28	
C_1	Control	7.00	7.00	6.75	6.50	6.25	6.7
	Raftiline	8.20	8.10	7.65	7.35	7.05	7.67
	Raftilose	8.10	8.00	7.55	7.25	7.00	7.58
	Oat fiber	7.10	7.10	6.79	6.55	6.20	6.74
	Honey#	7.82	7.82	7.45	7.10	6.83	7.22
	Raftiline +Oat	7.75	7.75	7.30	7.05	6.52	7.40
	Raftilose+ Oat	7.65	7.65	7.25	7.00	6.45	7.27
	Honey +Oat	7.88	7.88	7.31	6.95	6.72	7.35
Mean	-	7.68	7.66	7.25	6.96	6.62	-
C_2	Control	6.80	6.80	6.70	6.35	6.10	6.55
	Raftiline	7.88	7.88	7.55	7.25	6.85	7.48
	Raftilose	7.82	7.82	7.50	7.18	6.75	7.41
	Oat fiber	7.17	7.17	7.01	6.2	6.23	6.75
	Honey	7.71	7.71	7.42	6.89	6.56	7.25
	Raftiline +Oat	7.69	7.69	7.27	6.97	6.65	7.25
	Raftilose+ Oat	7.55	7.55	7.10	6.95	6.60	7.15
	Honey +Oat	7.75	7.75	7.40	7.00	6.70	7.32
Mean	-	7.54	7.54	7.24	6.84	6.55	-
C_3	Control	7.10	7.10	6.85	6.60	6.35	6.8
	Raftiline	8.25	8.25	8.00	7.79	7.15	7.88
	Raftilose	8.15	8.15	7.85	7.55	7.05	7.75
	Oat fiber	7.20	7.20	6.95	6.65	6.23	6.84
	Honey	8.00	8.00	7.76	7.28	6.95	7.60
	Raftiline +Oat	7.95	7.95	7.52	7.35	6.98	7.55
	Raftilose+ Oat	7.80	7.80	7.50	7.15	6.95	7.44

Table 4.45 Effect of bacterial culture, prebiotic ingredient and storage period on sensory quality (OAA) of yoghurt-cheese

Bacterial culture$(M)	Prebiotic Ingredient (S)	Sensory score (OAA) of yoghurt-cheese during storage					Mean
		Storage period in days (SS)					
		0	7	14	21	28	
	Control	7.00	7.00	6.75	6.50	6.25	6.7
	Raftiline	8.20	8.10	7.65	7.35	7.05	7.67
	Raftilose	8.10	8.00	7.55	7.25	7.00	7.58
C_1	Oat fiber	7.10	7.10	6.79	6.55	6.20	6.74
	Honey#	7.82	7.82	7.45	7.10	6.83	7.22
	Raftiline +Oat	7.75	7.75	7.30	7.05	6.52	7.40
	Raftilose+ Oat	7.65	7.65	7.25	7.00	6.45	7.27
	Honey +Oat	7.88	7.88	7.31	6.95	6.72	7.35
Mean	-	7.68	7.66	7.25	6.96	6.62	-
	Control	6.80	6.80	6.70	6.35	6.10	6.55
	Raftiline	7.88	7.88	7.55	7.25	6.85	7.48
	Raftilose	7.82	7.82	7.50	7.18	6.75	7.41
C_2	Oat fiber	7.17	7.17	7.01	6.2	6.23	6.75
	Honey	7.71	7.71	7.42	6.89	6.56	7.25
	Raftiline +Oat	7.69	7.69	7.27	6.97	6.65	7.25
	Raftilose+ Oat	7.55	7.55	7.10	6.95	6.60	7.15
	Honey +Oat	7.75	7.75	7.40	7.00	6.70	7.32
Mean	-	7.54	7.54	7.24	6.84	6.55	-
	Control	7.10	7.10	6.85	6.60	6.35	6.8
	Raftiline	8.25	8.25	8.00	7.79	7.15	7.88
	Raftilose	8.15	8.15	7.85	7.55	7.05	7.75
C_3	Oat fiber	7.20	7.20	6.95	6.65	6.23	6.84
	Honey	8.00	8.00	7.76	7.28	6.95	7.60
	Raftiline +Oat	7.95	7.95	7.52	7.35	6.98	7.55
	Raftilose+ Oat	7.80	7.80	7.50	7.15	6.95	7.44

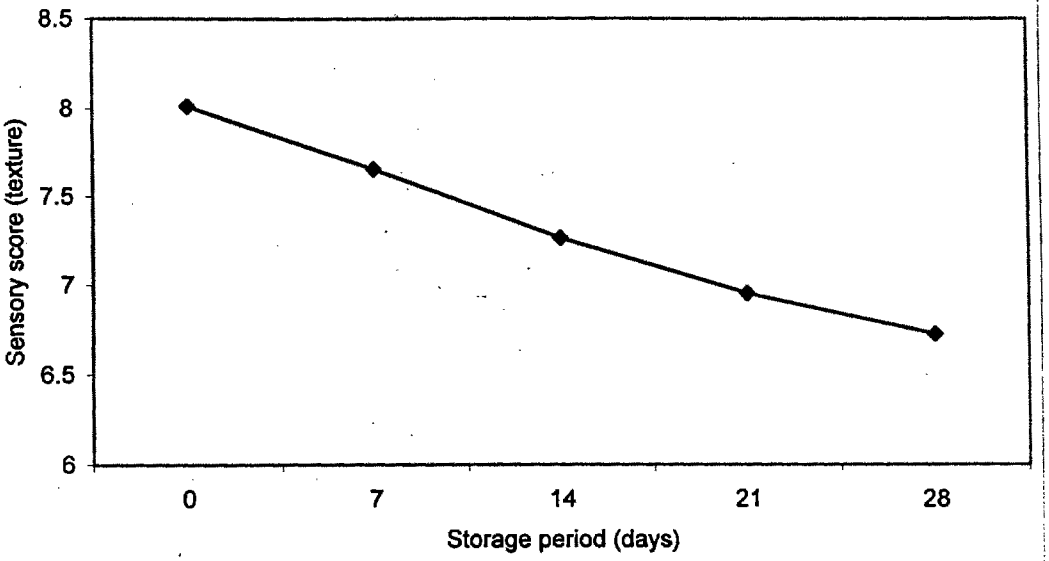

Fig. 4.39 Effect of storage period on mean sensory score for texture of yoghurt-cheese

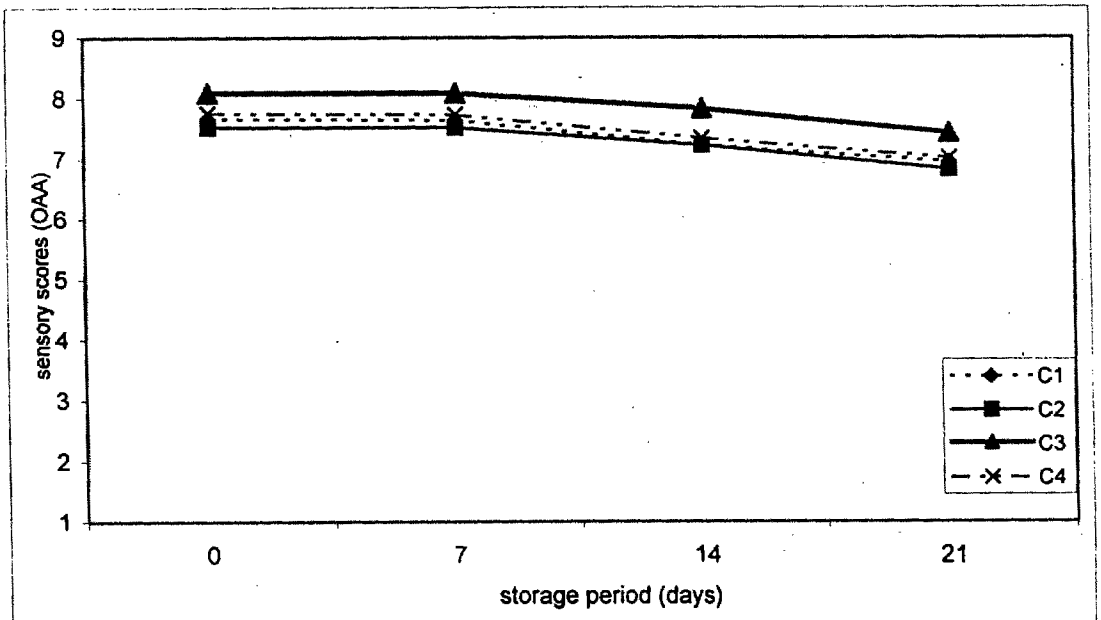

Fig.4.40 Effect of bacterial culture and storage period on mean sensory scores for overall acceptability (OAA) of yoghurt-cheese

C1= *L. bulgaricus+ S.thermophilus* (1:1)

C2= *L.bulgaricus+S.thermophilus+ L. acidophilus* (1:1:1)

C3= *L. bulgaricus+ S.thermophilus+B.bifidum* (1:1:1)

C4=*L.bulgaricus+S.thermophilus+ L. acidophilus+B.bifidum* (1:1:1:1)

highest overall acceptability followed by C_4, C_1 and C_2 cultures and the same trend was observed at all stages of storage.

Amiri (2001) also observed that the mean sensory scores for overall acceptability of yoghurt during storage remained unchanged till 11 days of storage and thereafter a gradual decrease was observed. She also reported that probiotic cultures caused a significant difference among the overall acceptability scores of yoghurt-samples. She obtained maximum sensory scores for yoghurt prepared by *B. bifidum* alongwith yoghurt starters and minimum scores for yoghurt prepared by yoghurt culture alone. Thus the results of present investigation are in fair confirmation to the above findings.

Summary & Conclusion

5. Summary and Conclusion

The present investigation was envisaged to optimize the parameters for the preparation of synbiotic yoghurt-cheese from standardized buffalo milk (0.5% fat). The effect of prebiotic/enriching ingredients namely raftiline, raftilose, oat fiber and honey used singly or in combination, on the physico-chemical and sensory characteristics of synbiotic yoghurt and yoghurt-cheese were studied. The probiotic cultures namely *Lactobacillus acidophilus* and *Bifidobacterium bifidum* alongwith traditional yoghurt cultures (*Lactobacillus delbrueckii* sub sp. *bulgaricus* and *Streptococcus salivarius* sub sp. *thermophilus*) were used in four different combinations for fermenting milk for yoghurt and yoghurt-cheese preparation. The yoghurt samples obtained were centrifuged at 9000 rpm for 15 min to obtain yoghurt-cheese. The starter cultures were also subjected to biocompatibility studies. The yoghurt-cheese samples were stored at 5±1°C for 28 days and the samples were analysed at an interval of 7 days for physico-chemical, microbiological and sensory characteristics to access the shelflife of the product. The specific findings of the investigation are as follows:

1. Buffalo milk containing 0.5 per cent fat, added with 2 per cent skim milk powder and 0.2 per cent sodium alginate gave a firm gel yoghurt after fermenting milk with traditional yoghurt cultures.

2. Heat treatment of standardized buffalo milk at 4 psi (~90°C) for 10 min was found optimum on the basis of sensory quality, which could be stored

satisfactorily at 5°C for 15 days. The open pan heating resulted in scum formation on the top of milk and more browning.

3. Optimization of prebiotic ingredients/enricher level in standardized milk was done on the basis of sensory characteristics namely colour, flavour, texture and overall acceptability of yoghurt. Raftiline, raftilose, oat fiber and honey were be satisfactorily added to milk for yoghurt preparation upto level of 4, 4, 1 and 3 per cent, respectively. Oat fiber at 0.5 per cent level could also be satisfactorily used along with 4 per cent raftiline or 4 per cent raftilose or 3 per cent honey. Soy protein isolate, orange and apple fibers, however, gave unacceptable product. Higher concentration of prebiotic ingredients/enricher masked the original desirable flavour of yoghurt.

4. The yoghurt prepared with or without addition of prebiotic/enriching ingredient to milk and fermented by different combinations of starter cultures were analysed for proximate composition. The moisture content of yoghurt ranged between 86.58 to 88.98 per cent and which was lower when prebiotic/enriching ingredients incorporated singly or in combination. However, all other proximate principles, namely protein, fat, ash and carbohydrate increased by incorporation of additives to the standardized milk due to corresponding decrease in moisture content. However, the effect of starter culture on proximate composition remained non-significant.

5. The pH and titratable acidity of yoghurt samples were found inversely related to each other. They remained unaffected by addition of prebiotic ingredient/enricher to standardized milk. However, the culture combinations used for fermentation of milk significantly ($P \leq 0.01$) affected the pH and titratable acidity of yoghurt. The highest decrease in pH (4.37) and increase in titratable acidity was observed in case of yoghurt prepared by C_4 culture which contained all four bacteria namely *L. bulgaricus, S. thermophilus, L. acidophilus* and *B. bifidum*, followed by C_2 culture having *S. thermophilus, L. bulgaricus* and *L. acidophilus*, C_1 culture having *S. thermophilus* and *L. bulgaricus* (control) and C_3 culture containing *S. thermophilus, L. bulgaricus* and *B. bifidum*.

6. The maximum yield of yoghurt-cheese (28.6%) along with optimal retention of total viable cells (~10^6 cfu/g) could be obtained by centrifuging yoghurt at 9000 rpm for 15 min. Centrifugation at higher speed however, resulted in cheese with low moisture content and mealy mouth feel.

7. The moisture content of yoghurt-cheese ranged between 70.20 and 73.44 per cent. Maximum moisture was present in control cheese samples and minimum in cheese prepared after addition of oat fiber to milk. Addition of prebiotic ingredients/enricher decreased the moisture content with corresponding increase in total solids content of synbiotic yoghurt-cheese significantly ($P \leq 0.01$). Highest protein (20.11%) fat (2.01%) and ash (1.13%) was observed in oat fiber added samples. Carbohydrate content

determined by difference (6.34%) was highest in yoghurt-cheese prepared from milk containing raftilose and oat fiber. However, the effect of culture combination on the proximate composition of cheese samples remained non-significant.

8. The pH and titratable acidity of synbiotic yoghurt-cheese samples remained unaffected by the type of additive used but the culture combination significantly altered them. Maximum acidity and lowest pH were observed in case of yoghurt-cheese prepared by C_4 culture followed by C_2, C_1 and C_3 culture. The range of pH and titratable acidity in yoghurt-cheese was 4.29 to 4.45 and 1.03 to 1.19 per cent respectively.

9. Whey obtained during centrifugation of yoghurt contained approximately 7.5-9.0 per cent total solids. The highest total solids were obtained in whey obtained from control yoghurt. The addition of prebiotic ingredients significantly ($P \leq 0.01$) reduced total solid content of whey. The proximate principles in whey namely protein and carbohydrate, however, increased significantly by incorporating prebiotic additives to milk.

10. The types of starter culture had significant effect on pH and titratable acidity of whey. The trends of changed in of pH and titratable acidity of whey were also similar those obtained for yoghurt and yoghurt-cheese.

11. All the four cultures were subjected to biocompatibility studies, which included changes in pH, titratable acidity during incubation at 42 ± 1^0C for 16 hours, bile tolerance and antagonistic activities towards pathogens.

Among four samples, yoghurt-cheese prepared by using C_4 culture (*L. acidophilus* and *B .bifidum* along with yoghurt cultures) had minimum pH (3.10) and maximum final titratable acidity (1.84%), followed by samples prepared by C_2 cultures (*L. acidophilus* along with yoghurt-culture), C_1 (yoghurt culture) and C_3 culture (*B. bifidum* along with yoghurt culture).

12. Incubation of yoghurt cultures with or without probiotic bacteria in lactic broth containing 0.0, 0.3 and 0.5 per cent bile salt (sodium glycolate) at 37°C for 16 hours revealed that all cultures having probiotic bacteria were more bile tolerant than yoghurt culture at both 0.3 and 0.5 per cent of bile concentration. The minimum inhibition of growth by 0.3 per cent bile salt was observed in C_4 culture (60%) having both probiotic bacteria namely *L. acidophilus* and *B. bifidum* along with yoghurt culture, followed by C_2 (63%) having *L. acidophilus* along with yoghurt culture, C_3 (64%) having *B. bifidum* along with yoghurt-culture and C_1 culture containing yoghurt starters alone (100%). Only C_4 and C_3 cultures could tolerate 0.5 per cent bile salt at the end of 16 h of incubation and the minimum and maximum inhibition of growth were observed in C_4 (68%) and C_1 (100%) cultures, respectively.

13. The studies on inhibition of human pathogens namely *Escherichia coli, Staphylococcus aureus* and *Salmonella havana* by cell free filtrates of starter cultures revealed that largest inhibitory zones were formed by C_3 filtrate against all the 3 pathogens followed by C_4 against *E. coli, S. aureus,* C_1 against *S. havana.* The minimum inhibitory zone against *E. coli* and *S.*

aureus was formed by C_1 filtrate and minimum zone against *S. harana* was formed by C_2 filtrate.

14. During storage of yoghurt-cheese at 5±1°C, it was found that the lowest pH and highest titratable acidity was obtained in case of cheese prepared using C_4 culture followed by those prepared by C_2, C_1 and C_3 culture . The pH and titratable acidity of the cheese samples respectively decreased and increased during storage period, respectively. The prebiotic/enriching ingredients had no effect on pH and titratable acidity of cheese. The maximum soluble nitrogen content (0.223%) was found in cheese samples prepared using C_1 (control culture) followed by C_3 (0.174%), C_4 (0.172%) and C_2 (0.164%) cultures. This indicated higher proteolytic activity in *L. bulgaricus* and *S. thermophilus* cultures in comparison to probiotic cultures. Among the additives, the combinations of oat fiber with raftiline, raftilose and honey gave higher soluble nitrogen content in cheese samples. The per cent soluble nitrogen content increased till 21 days of storage and then decreased slightly.

15. The total viable counts of cultures in yoghurt-cheese decreased during storage. The value of viable count of all samples remained within desirable limits for harvesting probiotic effects till 14 days of storage (2.43 to 2.99 x 10^6 cfu/gm). Samples prepared by C_4 and C_3 culture had maximum and minimum viable counts, respectively, but no significant difference in viable counts in cheese samples prepared by different cultures were

observed during storage. No yeast and mold count appeared till 14 days of storage and thereafter increased. The maximum and minimum yeast and mold counts appeared in cheese samples prepared by C_3 and C_2 culture respectively. The yeast and mold counts in samples remained within acceptable limits (<10 cfu/gm) till 21 days of storage. The proteolytic counts appeared after 7 days of storage and increased thereafter till end of storage. Highest proteolytic counts appeared in cheese sample prepared using C_1 culture followed by C_2, C_4 and C_3. Addition of prebiotic ingredients/enricher had no significant effect on microbial counts of cheese samples.

16. The type of starter culture had no effect on colour and texture of yoghurt-cheese. However, the flavour and overall acceptability of yoghurt-cheese were significantly ($P \leq 0.01$) affected by the starter cultures. Among all cultures, C_3 culture gave product with highest sensory scores, followed by C_4, C_1 and C_2. All the additives used, however, significantly ($P \leq 0.01$) affected the sensory scores for colour, flavour, texture and overall acceptability. The highest sensory scores were obtained by incorporation of 4 per cent raftiline, 4 per cent raftilose or 3 per cent honey singly or in combination with 0.5 per cent oat fiber. The sensory scores for overall acceptability remained in acceptable limit till 21 days of storage.

On the basis of above findings, it may be concluded that a satisfactorily good quality synbiotic yoghurt-cheese could be prepared from low fat buffalo milk

by incorporating 4 per cent raftiline or raftilose or 3 per cent honey, alone or in combination with 0.5 per cent oat fiber. The biocompatibility studies indicated that the probiotic culture namely *B. bifidum* had higher capacity for bile tolerance and antagonistic activity against food borne human pathogens namely *Escherichia* coli, *Staphylococcus aureus* and *Salmonella havana* followed by *L. acidophilus* than yoghurt cultures. The addition of *B. bifidum* as probiotic culture improved the sensory quality of the yoghurt-cheese significantly as compared to *L. acidophilus*. However, when both these cultures were used in combination with yoghurt culture, a synergistic effect on the physico-chemical and sensory quality of yoghurt-cheese was obtained. The yoghurt-cheese could be stored satisfactorily for 15 days under refrigeration and during this period, the viability of the probiotic cultures remained within desirable limits.

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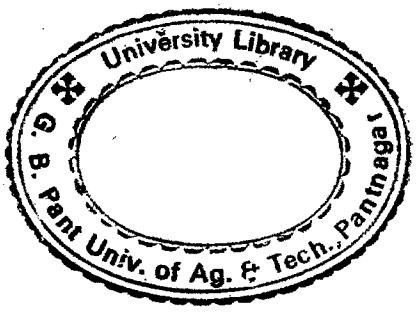

Appendices

Appendix I Analysis of variance for optimization of raftiline level in milk

Source of variation	df	Colour			Flavour			Texture			OAA		
		ss	ms	F	ss	ms	F	ss	ms	F	ss	ms	F
Treatment	5	0.025	0.005	ns	15.724	3.144	505.3**	17.997	3.599	459.63**	21.217	4.243	676.1**
Error	48	0.455	0009	-	0.298	0.006	-	0.375	0.007	-	0.310	0.006	-
Total	53	0481	-	-	16.022	-	-	18.373	-	-	21.519	-	-

Appendix II Analysis of variance for optimization of raftilose level in milk

Source of variation	df	Colour			Flavour			Texture			OAA		
		ss	ms	F	ss	ms	F	ss	ms	F	ss	ms	F
Treatment	5	0.032	0.006	ns	12.670	2.534	442.3**	20.731	4.146	464.78**	16.471	3.294	750.69**
Error	48	0.435	0.009	-	0.274	0.005	-	0.428	0.008	-	0.210	0.004	-
Total	53	0.467	-	-	12.945	-	-	21.159	-	-	16.682	-	-

Appendix III Analysis of variance for optimization of oat fiber level in milk

Source of variation	df	Colour			Flavour			Texture			OAA		
		ss	ms	F	ss	ms	F	ss	ms	F	ss	ms	F
Treatment	3	2.680	0.893	73.60**	2.928	0.976	188.50**	2.626	0.875	112.14**	2.721	0.907	140.97**
Error	32	0.388	0.121	-	0.165	0.005	-	0.249	0.007	-	0.205	0.006	-
Total	35	3.069	-	-	3.094	-	-	2.876	-	-	2.927	-	-

Appendix IV Analysis of variance for optimization of soy protein isolate level in milk

Source of variation	df	Colour			Flavour			Texture			OAA		
		ss	ms	F	ss	ms	F	ss	ms	F	ss	ms	F
Treatment	3	9.269	3.089	432.82**	6.534	2.178	302.73**	0.905	0.301	23.43**	3.360	1.120	107.08**
Error	32	0.228	0.007	-	0.230	0.007	-	0.412	0.012	-	0.334	0.104	-
Total	35	9.497	-	-	6.764	-	-	1.317	-	-	3.695	-	-

Appendix V Analysis of variance for optimization of honey level in milk

Source of variation	df	Colour			Flavour			Texture			OAA		
		ss	ms	F	ss	ms	F	ss	ms	F	ss	ms	F
Treatment	4	1.215	0.303	39.13**	6.923	1.730	269.02**	8.944	2.236	31584**	6.383	1.595	188.37**
Error	40	0.310	0.007	-	0.257	0.002	-	0.283	0.007	-	0.338	0.008	-
Total	44	1.526	-	-	7.180	-	-	9.228	-	-	6.722	-	-

Appendix VI Analysis of variance for optimization of raftiline and oat fiber level in milk

Source of variation	df	Colour			Flavour			Texture			OAA		
		ss	ms	F	ss	ms	F	ss	ms	F	ss	ms	F
Treatment	3	1.264	0.421	19.29**	5.767	1.922	138.95**	11.217	3.709	263.78**	8.769	2.923	257.79**
Error	32	0.699	0.021	-	0.442	0.013	-	0.450	0.014	-	0.363	0.011	-
Total	35	1.963	-	-	6.210	-	-	11.577	-	-	9.132	-	-

Appendix VII Analysis of variance for optimization of raftilose and oat fiber level in milk

Source of variation	df	Colour			Flavour			Texture			OAA		
		ss	ms	F	ss	ms	F	ss	ms	F	ss	ms	F
Treatment	3	1.590	0530	77.08**	5.169	1.723	94.49**	9.360	3.120	207.33**	8.726	2.908	277.20**
Error	32	.220	0.006	-	0.583	0.018		0.481	0.015	-	0.335	0.010	-
Total	35	1.810	-	-	5.752	-	-	9.842	-	-	9.062	-	-

Appendix VIII Analysis of variance for optimization of honey and oat fiber level in milk

Source of variation	df	Colour			Flavour			Texture			OAA		
		ss	ms	F	ss	ms	F	ss	ms	F	ss	ms	F
Treatment	3	1.423	0.474	49.66**	5.787	1.929	122.05**	8.736	2.912	171.53**	7.229	2.409	175.22**
Error	32	0.305	0.009	-	0.505	0.016	-	0.543	0.016	-	0.440	0.137	-
Total	35	1.729	-	-	6.293	-	-	9.279	-	-	7.669	-	-

Appendix- IX Analysis of variance for moisture, protein and fat content of yoghurt

Source of variation	df	Moisture			Protein			Fat		
		ss	ms	F	ss	ms	F	ss	ms	F
Bacterial cultures (M)	3	0.097	0.032	ns	0.034	0.011	ns	0.000	0.000	ns
Prebiotic ingredient (S)	7	16.312	2.039	130.5**	0.037	0.005	ns	0.000	0.000	ns
M*S	21	0.465	0.019	ns	0.109	0.005	ns	0.001	0.000	ns
Error	33	0.562	0.015	-	0.443	0.013	-	0.019	0.000	

Appendix- X Analysis of variance for carbohydrate and ash content of yoghurt

Source of variation	df	Carbohydrate			Ash		
		ss	ms	F	ss	ms	F
Bacterial cultures (M)	3	.007	.002	ns	0.004	0.001	ns
Prebiotic ingredient (S)	7	0.187	0.244	9.685**	0.027	0.003	ns
M*S	21	0.013	0.006	ns	0.043	0.001	ns
Error	33	0.088	0.002	-	0.066	0.008	-
Total	63	0.297	-	-	0.142	-	-

Appendix- XI Analysis of variance for pH and titratable acidity (% lactic acid) of yoghurt

Source of variation	df	pH			TA (% lactic acid)		
		ss	ms	F	ss	ms	F
Bacterial cultures (M)	3	1.467	0.489	154.47**	0.723	0.241	295.82**
Prebiotic ingredient (S)	7	0.040	0.005	ns	0.002	0.000	ns
M*S	21	0.047	0.001	ns	0.014	0.000	ns
Error	33	0.111	0.003	-	0.029	0.000	-
Total	63	0.669	-	-	0.770	-	-

**: Highly significant ns: Non-significant

Appendix- XII Analysis of variance for moisture, protein and fat content of yoghurt-cheese

Source of variation	df	Moisture			Protein			Fat		
		ss	ms	F	ss	ms	F	ss	ms	F
Bacterial cultures (M)	3	0.012	0.005	ns	0.175	0.586	ns	0.162	0.539	ns
Prebiotic ingredient (S)	7	12.314	3.140	122.3**	19.908	2.844	44.547**	0.200	0.028	9.211**
M*S	21	0.565	0.018	ns	1.072	0051	ns	0.011	0.00	ns
Error	33	0.487	0.012	-	2.043	0.064	-	0.099	0.003	-
Total	63	14.912	-	-	23.199		--	0.312	-	-

**: Highly significant ns: Non-significant

Appendix- XIII Analysis of variance for carbohydrate and ash content of yoghurt-cheese

Source of variation	df	Carbohydrate			Ash		
		ss	ms	F	ss	ms	F
Bacterial cultures (M)	3	0.009	0.003	ns	0.000	0.000	ns
Prebiotic ingredient (S)	7	12.565	1.795	9.685**	0.45	0.006	5.28**
M*S	21	0.021	0.001	ns	0.002	0.000	ns
Error	33	0.089	0.003	-	0.039	0.001	-
Total	63	12.685	-	-	0.087	-	-

**: Highly significant ns: Non-significant

Appendix-XIV Analysis of variance for pH and titratable acidity (% lactic acid) of yoghurt-cheese

Source of variation	df	pH			TA (% lactic acid)		
		ss	ms	F	ss	ms	F
Bacterial cultures (M)	3	0.358	0.119	1304.88**	0.073	0.024	68.232**
Prebiotic ingredient (S)	7	0.001	0.000	ns	0610	0872	ns
M*S	21	0.000	0.000	ns	0.235	0.112	ns
Error	33	0.003	0.000	-	0.115	0.000	-
Total	63	0.363	-	-	0.883	-	-

**: Highly significant ns: Non-significant

Appendix XV Analysis of variance for moisture, protein and fat content of whey

Source of variation	df	Moisture			Protein			Fat		
		ss	ms	F	ss	ms	F	ss	ms	F
Bacterial cultures (M)	3	4.250	1.146	ns	0.007	0.002	ns	0.000	0.000	ns
Prebiotic ingredient (S)	7	13.375	1.910	ns	0.128	0.018	12.052**	0.000	0.000	ns
M*S	21	32.750	1.559	ns	0.015	0.000	ns	0.000	0.000	ns
Error	33	48.625	1.519	-	0.0975	0.001	-	0.015	0.000	-
Total	63	99.000	-	-	0.249	-	-	0.015	-	-

Appendix- XVI Analysis of variance for carbohydrate and ash content of whey

Source of variation	df	Carbohydrate			Ash		
		ss	ms	F	ss	ms	F
Bacterial cultures (M)	3	0.002	0.004	ns	0.033	0.011	ns
Prebiotic ingredient (S)	7	10.254	1.587	**	0.091	0.013	-
M*S	21	0.025	0.000	ns	0.222	0.010	ns
Error	33	0.073	0.005	-	0.683	0.106	-
Total	63	10.354	-	ns	1.031	-	ns

**: Highly significant ns: Non-significant

Appendix-XVII Analysis of variance for pH and titratable acidity (% lactic acid) of whey

Source of variation	df	pH			TA (% lactic acid)		
		ss	ms	F	ss	ms	F
Bacterial cultures (M)	3	1.130	0.376	74.271**	0.005	0.001	25.836**
Prebiotic ingredient (S)	7	0.021	0.002	ns	0.000	0.000	ns
M*S	21	0.076	0.003	ns	0.001	0.000	ns
Error	33	0.324	0.005	-	0.004	0.000	-
Total	63	1551	-	-	0.883	-	-

Appendix- XVIII Analysis of variance for biocompatibility studies (pH and titratable acidity)

Source of variation	df	pH			Titratable acidity		
		ss	ms	F	ss	ms	F
Bacterial cultures (M)	3	0.161	0.054	105.87**	0.177	0.059	198.46**
Incubation period (S)	4	64.167	16.041	3147.82**	16.638	4.159	13955.5**
M*S	12	0.049	0.004	8.022**	0.054	0.004	15.105**
Error	40	0.020	0.000	-	0.011	0.000	-
Total	59	64.399	-	-	16.881	-	-

Appendix- XIX Analysis of variance for biocompatibility studies (bile tolerance)

Source of variation	df	Bile tolerance		
		ss	ms	F
Bacterial cultures (M)	3	0.985	0.328	451.95**
Incubation period (S)	4	5.037	1.259	1732.47**
Bile salt level (SS)	2	27.179	13.589	18693.49**
M*S	12	0.278	0.023	31.974**
S*SS	8	4.038	0.504	694.445**
M*SS	6	2.554	0.425	585.65**
M*S*SS	24	1.411	0.058	80.872**
Error	120	0087	0.000	-
Total	179	41.573	0.232	-

Appendix- XX Analysis of variance for changes in pH, titratable acidity and % soluble nitrogen during storage of yoghurt-cheese

Source of variation	df	pH			Titratable acidity			% Soluble nitrogen		
		ss	ms	F	ss	ms	F	ss	ms	F
Bacterial cultures (M)	3	1.603	0.534	11205.5**	1.285	0.428	8712.4**	0.078	0.002	2710.3**
Prebiotic ingredients (S)	7	0.000	0.000	ns	0.000	0.000	2.744*	0.751	0.000	111.49**
Storage period (SS)	4	10.506	2.626	55067.3**	6.409	1.602	32585.9**	0.103	0.025	2681.7**
M*S	21	0.004	0.000	4.13**	0.002	0.000	2.371**	0.000	0.000	1.836**
S*SS	28	0.004	0.000	3.480**	0.003	0.000	2.373**	0.000	0.000	ns
M*SS	12	0.233	0.019	408.35**	0.151	0.012	256.18**	0.000	0.000	146.034
M*S*SS	84	0.011	0.000	2.931**	0.007	0.000	1.779**	0.154	0.000	ns
Error	320	0.015	0.000	-	0.015	0.000	-	0.001	0.000	-
Total	479	12.380	0.025	-	7.875	0.01	-	0.209	-	-

**: Highly significant (at 1% level) *: Significant (at 5 % level) ns: Non-significant

Appendix- XXI Analysis of variance for sensory scores for colour and texture of yoghurt-cheese during storage

Source of variation	df	Colour			Texture		
		ss	ms	F	ss	ms	F
Bacterial cultures (M)	7	4.984	0.712	275.81**	2.415	0.034	19.052**
Storage period (S)	5	28.70	7.242	2805.2**	78.011	19.502	1076.59**
M*S	35	0.965	0.034	13.351**	2.610	0.093	5.145**
Error	320	0.826	0.002	-	5.796	0.018	-
Total	367	35.746	-	-	88.834	0.018	-

Appendix XXII Analysis of variance for sensory scores for flavour and overall acceptability of yoghurt-cheese during storage

Source of variation	df	Flavour			Overall acceptability		
		ss	ms	F	ss	ms	F
Bacterial cultures (M)	3	13.736	4.579	451.844**	14.750	4.916	278.38**
Prebiotic ingredients (S)	7	71.692	10.241	1010.64**	91.050	13.007	736.468**
Storage period (SS)	4	230.997	57.749	5698.62**	291.240	72.810	4122.52**
M*S	21	7.557	0.359	35.513**	0.816	0.038	2.201**
S*SS	28	3.557	0.127	12.538**	10.533	0.376	21.299**
M*SS	12	2.047	0.170	16.840**	0.895	0.074	4.226**
M*S*SS	84	5.400	0.064	6.344**	1.419	0.016	ns
Error	320	12..971	0.010	-	22.607	0.017	-
Total	479	347.960	0.241	-	433.312	0.301	-

Abstract

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A process was optimized to prepare synbiotic yoghurt-cheese from buffalo milk containing 0.5% fat by incorporating probiotics and prebiotics. On the basis of sensory analysis of yoghurt, it was revealed that the prebiotic ingredients namely raftiline, raftilose and enricher, honey could be satisfactorily added singly @ 4, 4 and 3 %, respectively or in combination with 0.5% oat fiber to milk. Starter cultures with or without probiotic bacteria were used @ 3% for the preparation of synbiotic yoghurt and yoghurt-cheese namely, yoghurt culture as control (C_1, 1:1), *L. acidophilus* with yoghurt culture (C_2, 1:1:1), *B. bifidum* with yoghurt starter (C_3, 1:1:1) and combination of *L. acidophilus* and *B. bifidum* with yoghurt culture (C_4, 1:1:1:1). The incubation was carried out at 42±1 ^{0}C. The addition of prebiotic ingredients/enricher had a significant ($P \leq 0.01$) effect on proximate composition of yoghurt, whereas, pH & titratable acidity (TA) of yoghurt were significantly ($P \leq 0.01$) affected by type of starter cultures. The highest decrease in pH (4.37) and increase in TA (0.88%) was obtained by C_4 culture, followed by C_2, C_1 and C_3 cultures. Yoghurt was centrifuged at 9000 rpm for 15 min to prepare yoghurt-cheese, which resulted in maximum cheese yield (28.6%) with minimum losses of viable counts in whey (2.1 x 10^2 cfu/ml). The moisture content in yoghurt-cheese ranged from 70.2-73.44% and prebiotic/enriching ingredients increased the protein, fat, ash and carbohydrate content of cheese samples. The minimum pH (4.29) and maximum TA (1.19%) was obtained by C_4 culture usage followed by C_2, C_1 and C_3 cultures. The whey contained 7.5 to 9.0 % total solids.

The biocompatibility studies of bacterial culture revealed that a synergistic effect existed between y *L. acidophilus* and *B. bifidum* as minimum final pH (3.10) and maximum final TA (1.84%) after 16 h of incubation at 42±1 ^{0}C was attained by C_4 culture. The probiotic cultures were found to be more tolerant to bile salt and the minimum inhibition of growth by 0.3 % bile salt was observed in C_4 culture (60%). Only C_4 and C_3 cultures could tolerate 0.5 % bile salt. The minimum and maximum inhibition of growth were observed in C_4 (68%) and C_1 (100%) cultures, respectively. Probiotic cultures also possessed higher antagonistic properties towards selected human pathogens namely *Escherichia coli, Staphylococcus aureus* and *Salmonella havana* than yoghurt culture. The largest inhibitory zones were formed by C_3 filtrate against all the 3 pathogens.

During storage of yoghurt-cheese for 28 days at 5±1°C, lowest pH and highest TA was obtained in cheese prepared using C_4 culture (3.74 & 1.66%). The pH decreased and TA increased through the storage of 28 days and were not affected by the additives used. Control cultures showed higher proteolytic effect than probiotic cultures. The total viable count of probiotic cultures remained with in desirable limits (2.43-2.99 x 10^6 cfu/g) till 14 days of storage. No yeast and mold counts and proteolytic counts appeared till 14 and 7 days of storage, respectively and thereafter increased. The sensory scores for colour, flavour, texture and overall acceptability of yoghurt-cheese remained acceptable till 21 days of storage. Satisfactory quality yoghurt cheese could be prepared by use of C_3 culture along with incorporation of raftiline (4%), raftilose (4%) or honey (3%) singly or in combination with oat fiber (0.5%).

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