DEVELOPMENT OF ENRICHED PROBIOTIC YOGHURT

NAGESH KUMAR, B.A.

DEPARTMENT OF DAIRY TECHNOLOGY

DAIRY SCIENCE COLLEGE, BANGALORE

KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR

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By

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CERTIFICATE

This is to certify that the thesis entitled ***"Development Of Enriched Probiotic Yoghurt"*** submitted by **Mr. Nagesh Kumar, B.A.,** in partial fulfillment of the requirement for the award of degree of **Master of Technology (Dairy Science)** in **Dairy Technology** to the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of bonafide research work done by his during the period of his study in this university under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
August, 2009

(Dr. B.V. VENKATESHAIAH)
Professor, Dean and Major Advisor

Approved by :

Chairman : _____
Dr. B.V. Venkateshaiah

Members : _____
Dr. H.M. Jayaprakasha

_____
Dr. Narasimha Murthy

_____
Dr. Arun Kumar, H.

_____
Dr. Manjunatha, H.

Affectionately Dedicated

To

My parents, Guide and Brother

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LIST OF ABBREVIATIONS

GRAS	-	Generally Regarded As Safe
Cfu/g	-	Colony-forming units/ gram
LDPE	-	Low Density Polyethylene
s	-	Seconds
h	-	Hours
g	-	Gram
SNF	-	Solids Not Fat
mM	-	milli molar
min	-	Minutes
mg	-	Milligram
BIS	-	Bureau of Indian Standards
^{0}C	-	Degree Centigrade
µl	-	Microliters
%	-	Per cent
%LA	-	Per cent of lactic acid
ml	-	Millilitre
hLF	-	human lactoferrin
LF	-	lactoferrin

INTRODUCTION

REVIEW OF LITERATURE

MATERIAL AND METHODS

EXPERIMENTAL RESULTS

DISCUSSION

SUMMARY

BIBLIOGRAPHY

ABSTRACT

I. INTRODUCTION

The annual milk production in India is estimated about 104.8 million tonnes in 2008 (www.nddb.com). About 55% of the milk produced in the country is being utilized for preparation of various indigenous dairy products like khoa and khoa based sweets, chhana and chhana based sweets, paneer, fermented milks etc. Fermented milk have been developed throughout the world as a means of preserving milk against spoilage.

The important fermented milks in India are dahi, Shrikhand, lassi, butter milk etc. These traditional foods have persisted over the centuries in the developing world. The scale of production ranged from house hold production to large scale production, wherein use of selected starter cultures, automatic processes and modern equipments are involved. Fermented milks are popular in view of organoleptic and other properties such as the characteristic flavour, refreshing taste and improved digestibility. The composition of fermented milks can be easily tailored to meet various dietary requirements especially in the production of low calorie fermented milks.

Yoghurt, the fermented milk is believed to possess special nutritional attributes even though complete supportive scientific evidence has been lacking. Its consumption seemed to be associated with population having greater longevity. Recent studies have provided evidence for beneficial role of yoghurt in human digestion and physiology *viz.* yoghurt culture produce certain metabolites during their growth in the product which aids the milk proteins to be digested and absorbed more rapidly than the native protein. Certain of these metabolites also have definite antagonistic effect against food borne

pathogens. It is also well supported that lactose intolerant individuals may be able to consume yoghurt without any adverse effect.

Even though yoghurt is similar to Dahi in its physical appearance, it posses excellent nutritional and therapeutic properties. There is a need to introduce yoghurt in the daily diet of Indians, especially with the incorporation of probiotic organisms. Yoghurt or yoghurt - like products have been used as the most popular vehicle for incorporation of probiotic organisms mainly *Bifidobacterium* and *Lactobacilli.*

Yoghurt is a fermented milk product produced from the usual yoghurt starter cultures, *Sterptococcus salivarius ssp. thermophilus* and *Lactobacillus delbrueckii ssp. Bulgaricus*. Over the years, research into yoghurt processes and ingredients has led to improvement in product quality and consistency. Cultures used have also been isolated, identified and maintained in culture collection centers throughout the world. With freeze drying technology these cultures can be preserved for easy transportation to any corner of the earth.

The term 'probiotic' is derived from Greek (word) meaning 'for life'. The probiotic foods are defined as "foods containing live microorganisms which actively enhance health of consumers by improving ecological balance of microflora in the gut when ingested in sufficient numbers" (Parker, 1974). It has been recommended that foods containing such bacteria should contain atleast 10^6 live organisms per gram of product at the time of consumption in order to produce therapeutic benefits. The consumption of probiotic products is helpful in maintaining good health, restoring vigour and combating intestinal and other disorders.

Whey is highly nutritious byproduct obtained during cheese/ paneer and casein manufacture. It comprises nearly about 50 % of nutritious milk solids that is 6-7 %, of which 14- 15 % protein, 70- 75 % lactose, 7- 8 % ash and water soluble vitamins (Renner, 1983). As per the FAO (2004) statistics 2,038,057 metric tonnes of dried whey is produced all over the world.

In India estimated whey production is 4.84 million tonnes (Aneja *et al.*, 2002). Among all the constituents of whey, whey proteins are the best proteins known. Among various sources of protein such as soya, beef, egg, casein etc, whey proteins are excellent both in terms of nutritional and economic point of view and also exhibits multidimensional functionality (Mann, 1998; Jayaprakasha and Brueckener, 1999).

Nutritional value of WPC is mainly dependent on higher concentration of essential amino acids, such as tryptophan, leucine, isoleucine, threonine and lysine. The nutritional and functional properties of WPC are well documented. Because of their excellent nutritional and functional properties,

In view of the changing trend of consumption that has apparently justified man's endeavour in evolving and exploiting newer product concept, this investigation had been made to develop enriched probiotic yoghurt by replacing MSNF with whey protein concentrate (WPC) and addition of probiotic organisms and fruit pulp. This may help to upgrade and improve the fermented milk market in India. Consumption of yoghurt not only improves the nutritional status but also posses the therapeutic properties. In this context development of enriched probiotic yoghurt adds to the economic development of

Indian dairy industry. With all these considerations the present investigation was carried out with the following objectives;

1. To study the effect of different levels of probiotic cultures on the quality of yoghurt.

2. To optimize the processing parameters such as Heat treatment on the quality of probiotic yoghurt.

3. To study the effect of different levels of WPC and fruit pulps on the quality of enriched probiotic yoghurt.

4. To study the shelf stability of probiotic enriched yoghurt by using different packaging materials.

II. REVIEW OF LITERATURE

2.1 History of yoghurt :

Acidification of milk by fermentation is one of the oldest methods of preserving milk and conferring special organoleptic qualities to the product. Fermented products vary considerably in composition, flavor and texture according to the nature of fermenting organisms, the type of milk and the manufacturing process. Cultured milk products, which include yoghurt, cheese, lassi and shrikhand plays an important role in human diet. The low pH and extended shelf life make cultured milk products practically relevant to commercial production in subtropical countries. The sensory attributes are very important determinants for the acceptability of fermented dairy products (Vedamuthu, 1991).

The term yoghurt is derived from the Turkish word "Jugurt". It is called by various names in different parts of the world. For instance in India it is known as Dahi or Dadhi or Dahee. In Egypt & Sudan it is known as Zabady (Tamine and Deeth, 1980). Origin of yoghurt could be traced to the Middle East and the evolution of this fermented product through the ages can be attributed to the culinary skills of the nomadic people living in that part of the world (Tamine and Robinson, 1985).

The process of yoghurt making is an ancient craft, which dates back to thousands of years, but it is assumed that prior to nineteenth century, the various stages involved in the production of yoghurt were little understood. The uniqueness of yoghurt is attributed to symbiotic fermentation (Vedamuthu, 1991). Use of fermented / cultured dairy products

have been the essential part of our food consumption. Since ancient times, conversion of milk into cultured dairy products by souring with appropriate microbial inoculation is common practice in every house hold. Yoghurt, a traditional milk product of the Middle East countries is relatively a new introduction to Indian dietary system (Thompkinson and Sahal, 1995).

2.2 Definition

Yoghurt can be defined as a cultured milk product obtained by using starter cultures such as *Streptococcus salivarius ssp. thermophilus* and *Lactobacilllus delbrueckii ssp.bulgaricus*. The product should contain 0.8% lactic acid, while Yeast and Mold counts not exceeding 100 per gram and Coliform count not more than 10 per gram. The product should be negative for phosphates test (IS 7035, 1973).

According to International Dairy Federation (IDF, 1969), yoghurt may be defined on the basis of source of milk *viz.* Cow, goat, sheep and buffalo, processing condition, fermentation process using specific micro-organism and IDF suggest a minimum of 0.7% lactic acid content in retail product. Yoghurt also defined as the solid, custard like fermented milk product made from fortified high solids milk using symbiotic mixture of *Streptococcus salivarius ssp. thermophilus* and *Lactobacillus delbrueckii ssp.bulgaricus* as starters (Vedamuthu, 1991).

According to FAO / WHO (1976) "Yoghurt is a coagulated milk product obtained by lactic acid fermentation of milk through the action of *S.salivarius ssp. thermophilus* and *L.delbrueckii ssp. bulgaricus* with or without addition of whole milk powder or whey solids. The desirable micro organisms in the final product must be viable and abundant.

2.2.1 Composition

Yoghurt is produced by culturing cream, milk, partially skimmed milk or milk either alone or in combination with lactic acid producing bacteria viz, *L. bulgaricus* & *S. thermophilus*. The regulations specify that yoghurt before addition of bulky flavors contains not less than 3.25% milk fat, 8.25% MSNF and titrable acidity of 0.9% lactic acid (Vedamuthu, 1991).

The concentration of milk solids in most of the commercial yoghurt made from whole milk ranged from 14 to 16%, while in yoghurt made from skimmed milk ranged between 9 to 10% (Kulkarni, 1990). An increase in total solids results in the increase in the titratable acidity and reduction in coagulation time.

2.2.2 Nutritional and Therapeutic properties of Yoghurt

Yoghurt is highly recommended to the lactose intolerant individuals because of the reduced lactose content. Besides this, lactic acid also helps in the absorption of calcium and phosphorous in the intestine (Renner, 1986). It is an excellent source of protein and meets the minimum daily protein requirement of an adult and this protein is highly digestible as most of the protein is in the pre-digested form (Tamine and Deeth, 1980). Further, consumption of yoghurt promotes growth as a result of improved lactose digestion, greater mineral absorption besides providing thiamine, riboflavin, niacin and folic acid (Renner, 1986).

The more recent studies revealed that yoghurt cultures are capable of controlling intestinal disorders such as diarrhoaea and constipation. *L.bulgricus* can inhibit intestinal

putrefaction and can be effective in treating gastro-intestinal disorders (Tamine and Deeth, 1980). Further yoghurt cultures are capable of controlling blood cholesterol (Rao *et al.*, 1994).

2.2.3 Classification of yoghurt

At present there are different types of yoghurt produced worldwide. Tamime and Deeth (1980) proposed four categories based on the physical characteristic of the product. However, these products and in particular yoghurt are subdivided into different groupings based on the following aspects:

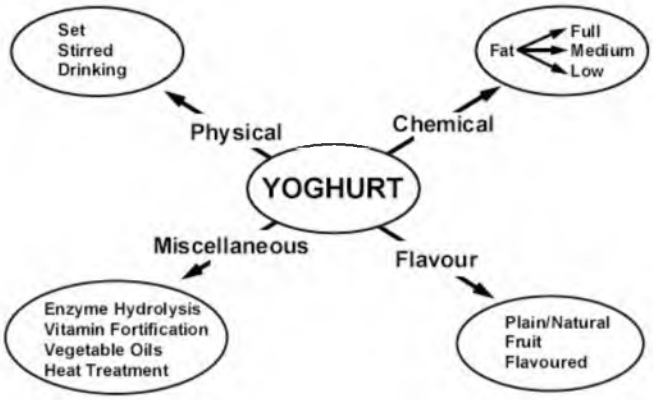
- Legal standards (i.e. existing or proposed) to classify the product on the basis of chemical composition or fat content (full, semi skimmed/medium or skimmed/low fat).

- Physical nature of the product, i.e. set, stirred or fluid/drinking; the latter is considered stirred yoghurt of low viscosity.

- Flavours (plain/natural, fruit or flavoured; the latter two types are normally sweetened).

- Post-fermentation processing (vitamin addition or heat treatment).

Classification of all yoghurt products



Adopted from Tamine and Deeth(1980) and Tamine and Marshal (1997)

Fig. Schematic representation for the classification of yoghurt.

2.4 Effect of processing variables on quality of Yoghurt.

2.4.1 Homogenization

Homogenization is an integral part of yoghurt manufacturing process. It is usually carried out before the final heat treatment (Kulkarni, 1990). Milk used for yoghurt is a typical oil in water type of emulsion, which has tendency to separate out on standing especially during incubation period in order to prevent this and also to have a uniform consistency throughout, the yoghurt mix is homogenized to breakup the fat globules. The homogenization reduces the average diameter of the fat globules to less than the size of two micron and prevents cluster formation and tendency for fat to rise to the surface (Chawla, 1985; Tamine and Robinson, 1985).

Homogenization improves the consistency and viscosity of yoghurt mainly because of mechanical incorporation of the finely divided globules within the coagulum structure. Homogenization pressure of 2500 psi in first stage and 500 psi in second stage was recommended for buffalo milk yoghurt with a minimum whey separation (Chawla, 1985). Acidity of homogenized milk coagulum was higher and increased with increased homogenization pressure (Volkova and Radulov, 1986).

Homogenization temperature (50^0 or 60^0C) or homogenization pressure (5-20 MPa) had no major effect on viscosity of drinking yoghurt. Variation in the sequence of homogenization after or before pasteurization did not effect on the stability of the product (Hooydonk *et al.*, 1984). Application of homogenization process in the production of two types of yoghurt (3.5 and 5.0% fat) resulted in reduced wheying off, increased firmness and also extended storage period by 70-160% (Szakaly *et al.*, 1986). Homogenization

pressure of 200kg/cm^2 was found to provide yoghurt with a firm body and smooth texture compared to 100, 150 and 250 Kg/cm^2 (Venkateshaiah, 1995). Single stage with 2000 psi pressure was found to provide yoghurt with firm body and smooth texture (Chidanand, 2003).

2.4.2 Heat treatment

The primary aim of thermal processing in the manufacture of the yoghurt is for the destruction of micro organisms which may be pathogenic or which may adversely affect the quality of yoghurt (Vedamuthu, 1991)

Casein constitutes the major group of proteins in milk, which play an important role in the manufacture of yoghurt. The other types of proteins are serum or whey protein, which will have more defined, compact, globular shape than the caseins. The functional properties of this whey protein become more apparent after heating of the milk to more than 80^{0}C. At this temperature protein are denatured and bind with α-casein to form more stable micelles with optimum hydrophilic properties. Hence firm coagulum of the yoghurt mix is obtained by heating milk to 80^{0}C for 30 min (Gregerov, 1986).

The heating of milk above 80^{0}C results in denaturation of whey proteins. The maximum hydration of proteins occurs when the milk is heated to 85^{0}C. These hydrophilic properties of proteins can affect the syneresis characteristics of yoghurt. Hence in order to produce yoghurt with minimum syneries, the whey protein denaturation should be in the range of 70 to 90% (Kulkarni, 1990).

Heat treatment of 85^0C for 30 min was found to be most favorable in yoghurt prepared from modified milk containing groundnut protein isolates and buffalo milk (Venkateshaiah *et al.*, 1995). Heating at 85^0C for 5 min was sufficient to produce good quality yoghurt from buffalo milk containing whey protein concentrates (Mohammed *et al.*, 1991). Yoghurt milk fortified with skim milk powder heated to 95^0C for 5 min contributed higher viscosity compared to milk exposed to 65^0C for 15 min (Thomopulous *et al.*, 1993). The rate or reduction in viscosity was higher in plain liquid yoghurt obtained form UHT treated milk (145^0C/5 sec) than vat pasteurized milk (Hooydonk *et al.*, 1984).

2.4.3 Yoghurt culture and their associate growth

Yoghurt is a fermented dairy product produced by the symbiotic microbial action of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The observation of symbiotic relationship between these two micro organisms was first reported by Orla Jenson (1931). *Lactobacillus delbrueckii ssp bulgaricus* is the new nomenclature for *L. bulgaricus*. Because of the high phenotypic and genomic similarities between *L. delbruckii, L. bulgaricus and L. lactis, L. delbruckii* is retained and the other two are designated as subspecies (Marshall, 1987).

Streptococcus thermophilus was reclassified because of the close nucleic acid homology and similar profile of long chain fatty acid with the species *S. salivarius ssp. thermophilus* is paired with proteolytic partners, example *L. delbrueckii ssp. bulgaricus* for milk fermentation. All strains of the species grow well at 45^0C and some could grow

between 48^0C to 52^0C. Subspecies *bulgaricus* ferments only glucose, fructose and lactose (Marshall, 1987).

Foster *et al.* (1957) recommended the use of 1.5% inoculation and an incubation temperature of 43^0C for 4 hrs for preparation of yoghurt. The culture used significantly influences the quality of yoghurt. Tamine and Robinson, (1989) reported the use of 2% mixed culture of *Streptococcus* and *Lactobacillus* in the ratio of 3:1 to get good quality yoghurt with pleasant flavour. Madanlal *et al.*, (1978) and Goh *et al.*, (1982) suggested the combinations of 1:1 to 1:2 of *Streptococcus* and *Lactobacillus* for getting the desirable characteristics of yoghurt. Chidanand (2003) reported that the use of 2% level of inoculum in egg white based yoghurt.

2.5 Whey Protein Concentrate (WPC)

Whey is highly nutritious byproduct obtained during cheese / paneer and casein manufacture. It comprises nearly 50% of nutritious milk solids that is 6-7%, of which 14-15% protein, 70-75% lactose, 7-8% ash and water soluble vitamins (Renner, 1983). As per the FAO (2004) statistics 2,038, 057 metric tonnes of dried whey is produced all over the world. India's estimated whey production is 4.84 million tones (Aneja *et al.*, 2002). Among all the constituents of whey proteins comprises of 61.4% β-lactoglobulin, 20.5% α-Lactalbumin, 60% serum albumin and 12.2% immunoglobulins. Whey proteins are one of the highest quality natural proteins available (Patel *et al.*, 1993) and their nutritional value is depending on higher concentration of essential aminoacids such as tryptophan, leucine, isoleucine, threonine and lysine than casein (Renner, 1983).

WPC represents a protein source of excellent nutritional importance. WPC and isolates are generally recognized as safe (GRAS). There has been a significant increase in WPC production since the introduction of UF processes (Gupta and Thapa, 1991).

2.5.1 Nutritional and functional properties of WPC

Among various sources of protein such as soya, beef, egg, casein etc, whey proteins is excellent both in terms of nutritional and economic point of view, it also exhibits multidimensional functionality (Mann, 1998: Jayaprakasha and Brueckner, 1999). The WPC contains β-lactalbumin (21%), serum albumin (5%), Immuno-globulins (10%), Proteose peptone (10%) (Huffman, 1996). In addition, it contains milk enzymes such as lysozyme, lipase and xanthine oxidase, which are present in low concentrations (Bajaj *et al.*, 2002).

Nutritional value of WPC is mainly dependent on higher concentration of essential aminoacids, such as tryptophan, leucine, isoleucine, threonine and lysine. It contains total of 50.9g of essential aminoacids for every 100g protein, where as casein contains only 45.1 g/100g of protein (Renner, 1983). The sulphur amino acids particularly 2.5g of cystiene and 2.8g of cystine per 100g protein are higher in whey protein than in casein (Irvine *et al.*, 1984). Whey proteins have higher biological value (104) as against whole egg protein (100) and casein (77). They posses higher protein efficiency ratio (PER) and net protein utilization (NPU) than casein. The PER of whey protein is 3.6 whereas 3.8 for whole egg protein and 2.9 for casein, where as NPU is 93 for whole egg, 76 for casein and 95 for whey proteins. The whey protein requirement of an adult of average body weight 70kg could be furnished by consuming only 14.5g of

whey proteins as against 28.4g of cow milk protein (or) 17.5 g of egg protein (Renner and Abd-El- Salam, 1991).

Whey proteins are also known to exhibit high anticarcinogenic activity than other dietary proteins like meat and soya, which may be related to their sulphur amino acid content (Mc Intosh *et al.*, 1998).

Functional properties of proteins in food systems are those physico-chemical properties, which provide the desired organoleptic (or) textural characteristeics on the product in which they are used (Dewit, 1984). Whey proteins could be used as a component of foods for its functional, nutritional and economic benefits (Rakashy, 1988; Giese, 1994).

Whey protein products are more stable to pH changes and the protein composition influences the functional properties of WPC (Kim and Gilliland., 1989). Heating of whey proteins causes a slight increase in viscosity and water holding capacity (Huffman, 1996). Apart from nutritional aspects whey proteins are used as potential functional ingredients in various food formulations.

2.5.2 Application of WPC

2.5.2.1 Dairy products

During past two decades the conservation of whey solids in the form of WPC has gained importance and the increased production of WPC has warranted its application in various food products (Gupta and Thapa, 1991). Several dairy products such as ice-

cream, yoghurt, rosogolla etc. have been formulated by incorporating WPC (Jayaprakasha, 1998)

Utilization of WPC in the manufacture of indigenous dairy products is still at its infancy. WPC was successfully incorporated upto a level of 11% into khoa during its preparation without any adverse effect on organoleptic and shelf life of khoa (Muralidhara, 1989). Gulab jamoon prepared with 10% WPC, showed better sensory quality with respect to colour and appearance, body and texture, flavour, juiciness and overall acceptability (Devaraja, 2005).

Use of WPC and SMP in the ratio of 40:60 resulted in good quality Gulab jamoon and was comparable with the control samples (Vani Rai, 2000). Blending WPC (70%) at 5% level in the preparation of rosogolla without affecting any physico-chemical and sensory attributes has been reported by Suma (2002). Sandesh prepared by incorporating 10% WPC secured highest sensory scores with respect to colour and appearance, body and texture, flavour and overall acceptability (Mahadevaiah, 2004).

Attempts have been made to utilize WPC and butter milk solids either alone or both in combination with skim solids in various low fat, non fat frozen dairy products and ice cream (Opdahl and Bear, 1991; Thirumalesha and Jayaprakasha, 1998). Naidu *et al.*, (1986) observed a decrease in viscosity of ice cream mix and increase in acidity when whey solids levels were increased.

Replacement of skim milk powder with WPC at the rate of 50% into ice cream mix resulted in improved flavour, body and texture and melting quality of ice cream

(Hugunin, 1987). WPC addition was found to improve the nutritive value of the products and it can be used in whipped toppings and creams (or) foamy products (Huse, *et al.*, 1984).

Kulfi prepared by replacing skim milk solids to an extent of 80% with WPC, resulted a product with higher overrun, better mouth feel and improved acceptability than standard kulfi (Jayaprakasha and Brueckener, 1999).

2.5.2.2 Cheese

The cheese industry has shown considerable interest in incorporating WPC. WPC has been successfully utilized in the manufacture of cheese spread and cheese foods (Irvine *et al.*, 1984). An acceptable quality processed cheese food was prepared by replacing 20% of cheese solids of cheddar cheese with that of WPC (Thapa and Gupta, 1992).

Improved sensory scores were observed for cheese added with WPC at a rate of 10% to milk and coagulation carried out using calf or microbial rennet (Santoro, 1994). Improved viscosity was noticed in double cream cheese containing whey protein concentrate (Sanchezt *et al.*, 1996).

2.5.2.3 Yoghurt

Attempts have been made to utilize WPC in yoghurt and frozen dessert (Jelen *et al.*, 1987; Gupta and thapa, 1991; Venkateshaiah, 1995 and Jayaprakasha, 2000). Yoghurt with WPC upto 20% was found to be firmer with less syneresis (Jelen *et al.*, 1987). The

yoghurt was found to have less curd tension with increase in WPC (Mohammed *et al.*, 1991).

WPC was incorporated to buffalo milk upto 20% in yoghurt manufacturing with improved texture, mouth feel and reduction in wheying off (Renner and Abd-El-Salam., 1991). The viscosity and stability of yoghurt improved by addition of WPC-35 to yoghurt mix. A combination of whey protein and heat treatment has created the desired water holding effect and increased viscosity without any syneresis (Barrentes *et al.*, 1994).

Frozen yoghurt could be prepared successfully by replacing 50% of skim milk solids with WPC (35%). The product obtained had better body and texture and resembled the consistency of ice cream (Opdhal and Baer, 1991; Jayaprakasha, 2000).

Yoghurt prepared by replacing 25% non-fat dry milk with WPC had acceptable quality and it was concluded that the body characteristics of yoghurt can be readily controlled by giving proper heat treatment to whey proteins at an elevated temperature. An increased sensory score was noticed in yoghurt manufacture by the addition of 15 to 40% WPC (Abdul Rabo *et al.*, 1987). Reddy *et al.*, (2005) supplemented that the addition of WPC @ 0.5% to the yoghurt milk can help in improving the viability of *L. acidophilus* without affecting the overall acceptability of yoghurt during storage at 4^0C.

2.6 Probiotic cultures

Parker (1974) introduced the term "probiotic" to describe "organisms and substances which contribute to intestinal microbial balance". Probiotics are the live microbial feed supplemented, which beneficially affects the host animal by improving the

intestinal microbial balance (Fuller, 1989). 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 the colon" (Ziemer and Gibson, 1998). Synbiotics are the mixtures of both probiotics and prebiotics (Gibson and Roberfroid, 1995). The mix would benefit the host by improving survival and implantation of the selected microbial supplements.

Very large populations of bacteria reside in the small and especially the large intestine of human. In healthy subjects they are considered the normal intestinal microflora (Mitsuoka, 1989). Recent research has shown that the genus *Bifidobacterium* is predominant in a well – balanced intestinal microflora of healthy individuals and this is especially true in infants (Gilliland, 1989). The foods containing Bifidobacteria and Lactobacilli have become more popular and global market demand for these products has increased sharply.

2.6.1 *Lactobacillus acidophilus.*

Acidophilus cultures have been widely used either through fermented milk or in the form of all concentrates to treat gastro-intestinal disorders and other ailments in human beings and livestock and to help restoring normal intestinal flora after antibiotic treatment (Prabha, 1984).

L. acidophilus cultures have been shown to be inhibitory towards many of the commonly known food borne pathogens (Lim *et al.*, 1993). Kim and Gilliland (1983) investigated the effect of *L. acidophilus* as a dietary adjunct in milk to aid lactose digestion in humans. It produces β-D galactosidase enzyme and hydrolyze the lactose,

which results to intra-intestinal digestion of lactose. Kiyoe *et al.*, (1986) reported that the freeze-dried culture of *L. acidophilus* prevents the increase of blood cholesterol, after eating foods such as butter or eggs with high cholesterol content.

L. acidophilus reduces the cholesterol due to a co-precipitation of cholesterol with deconjugated bile salts at lower pH values as a result of lactic acid production by bacteria (Kailaspathy and Chin 2000). The anti-tumor action of *L. acidophilus* is attributed to the inhibition of carcinogens and procarcinogens. Inhibition of bacteria that convert procarcinogens to carcinogens (Gilliland, 1989).

2.6.2 *Bifidobacterium bifidum*

Bifidobacteria were isolated and described in the period of 1899-1900, two type species *Bacillus bifidus*-communis or *B. bifidus*. This organism was an anaerobic G+ve, curved, rod, often occurring as bifurcated Y-form. It is the predominant organism in the stools of breast fed infants (Buchanan and Gibbons, 1974). Regular consumption of dietary supplements of Bifidobacteria and Lactobacilli may mitigate certain undesirable effects of some components of the gut flora. For instance Bifidobacteria prevented the formation of amines in the gut (Dave and Shah, 1998). A mixture of Bifidobacteria and Lactobacilli was grown in milk containing various additives by incubating for 18 to 20 hrs at 37^0C. The resultant product was effective in the treatment of gastro intestinal diseases in children (Doro *et al.*, 1983).

The consumption of milk containing Bifidobacteria improved the bowel movement in the adults, decreased pathogens in bowels and enhanced resistance to cancer and liver fucctio (Yuguchi *et al.*, 1987) and also reduced dimethylnitosamine

levels in mammals. It is used as a preventive medicine for cancer of the digestive tract (Kawai *et al.*, 1987). *Bifidobacteria* can be used to control enteric infections, lower serum cholesterol levels. *Bifidobacteria* improve the infant and milk products more nutritious and more easily digestible be lactose intolerant people (Laroia and Martin, 1990).

Kurmann and Rasic (1991) reported that Bifidobacteria in the intestines of infants and young children give protection against invading pathogens by production of organic acids and other antimicrobial substances and in adult intestine they may inhibit tumour growth and stimulate the immune system.

Bifidobacteria produce both lactic and acetic acids but higher amounts of acetic acid are produced which exhibits a stronger antagonistic effect against gram negative bacteria than lactic acid bacteria (Rasic and kurmann 1983). Scherbakova *et al.*, (1990) reported that use of Lysozyme and Bifidobacteria increased the effectiveness of the treatment, promoted weight gain and increased resistance to infection. Lysozyme increased the adhesive properties of Bifodobacteria *in –vitro*.

2.6.3 Fermented products containing Bifidobacteria and Lactobacilli

Rossi and Costamagna (1977) cultured four different type of milk (Soya milk, ultra filtered milk, partially delactosed milk and cow's whole milk) with *L. acidophilus, L. bulgaricus, S. thermophilus* and *B. bifidum*. The fermented milks were then stored at 8-10^0C for up to 15 days. Counts of *L. acidophilus* and *B. bifidum* remained at about 10^7 cfu/ml. while *L. bulgaricus* and *S. thermophilus* declined to about 104 cfu/m in low-lactose milk and more rapidly in Soya bean milk.

Costamagna and Rossi (1980) developed a beverage from milk with the addition of ultra filtered whey protein concentrates and inoculating with *L. acidophilus, B. bifidum* or yoghurt cultures and by incubating at 43^0C. Mudai *et al.*,(1982) prepared cultured milk using *B. bifidum* and a slow acid producing *L. bulgaricus* or *L. acidophilus* under aerobic conditions without growth promoters.

A new protein-enriched cultured milk product "cultura" and a skim milk "cultura drink" both containing large numbers of *B. bifidum* and *L. acidophilus* were prepared and the product had a taste similar to yoghurt Taylor (1987). The best quality cottage cheese could be prepared using bifidobacteria together with mesophillic starter cultures (Borisova, 1987).

Mathur (1993) reported that *bifidus* containing infant formula was capable of retaining 1.2 x 10^5 cfu/gm of viable *B. bifidum* cells upto 12 months of storage under ambient conditions and shown a close similarity to human milk and superiority over common available commercial formula. Commercial low fat ice-cream produced utilizing a practical form of direct frozen culture addition, retains levels of viable probiotic organisms above 1.0 x 10^6 cfu/gm over an extended shelf life period (Haynes and Playne, 2002).

Shiva Prakash (2002) developed a filled probiotic Kulfi by inoculating *L. acidophilus* and *B. bifidum* cells and then incubated at 37^0C for 5 hrs, hardened and then made available as a frozen dairy dessert rich in live cells of *L. acidophilus* and *B. bifidum.* Christopher *et al.,* (2006) developed probiotic yoghurt using *B. bifidum* supplemented with 0.5% whey protein concentrates which helped maintain viability of *B.*

bifidum strains without affecting the overall acceptability during storage at 4^0C for at least 4 weeks.

2.7 Fruit pulps

Different fruits are used for enriching the fermented milks. A variety of different fruits are currently used to improve the marketability of yoghurt (Valenzkey, 1978; Vance Grosser, 1978). Fruit pulps have been used in the manufacture of frozen yoghurt at a level of 10 to 14% to improve overall acceptability of the product (Knupp, 1979). Collier and Cardwell (1988) prepared frozen soft serve yoghurt by blending yohgurt with ice cream mix in the ratio of 40:60 with 10% grape puree (Vit. Rotondifolia). Sensory evaluations indicated that these products had acceptable flavour.

The effect of fruit and its level of concentration was found to be non significant on appearance, aroma, taste and overall acceptability of the product. The highest acidity (1.01% lactic acid) was recorded incase of yoghurt blended with 10% banana pulp. (Nila *et al.*, 1987).

Addition of mango fruit in yoghurt has been recommended by Ajam *et al.*, (1993). Among pineapple, mango, banana and sapota fruit pulps tried at different levels with frozen yogurt, mango fruit pulp at 10% was found to give a highly acceptable product compared to other fruit pulps (Venkateshaiah, 1995).

Fruit flavoured yoghurt drinks were acceptable to a major segment of the consumers to single strain starter which produced 0.73 to 0.86% lactic acid and 1.5 to 1.9 ppm of acetaldehyde in skim milk based yoghurt (Ashour, *et al.*, 1985).

2.8 Storage stability

The viscosity of yoghurt with Ewe's milk was found to decrease considerably during storage. Where as viscosity of yoghurt made from goats or cow milk remained same or tended to increase slightly (Kehagias *et al.*, 1985). The degree of microbial contamination of yoghurt with coliforms remained less than 10 per ml for two week storage at 7^0C, yeast and mold counts were found to increase from 1 cfu/ml to 22 cfu/ml after 2 weeks of storage (Salji *et al.*, 1987). Yoghurt made from vat pasteurized milk had better keeping quality (4 weeks) than UHT processed milk (2 weeks) (Salji, *et al.*, 1984).

Carbonation at 0.5% / kg/cm^2 retard acid development in sweetened plain yoghurt beverage during cold storage with excellent quality being maintained for 4 months at 4.4^0C. Whereas non-carbonated controls were found to be unacceptable within 30 days (Choi and Kosikowski, 1985). Brabrand factory in Denmark has developed various type of flavoured yoghurt drink packaged in gable top one liter cartoons and claim a keeping quality of 19 days (Anonymous, 1986b). Yoghurt drink that was heat shocked at 60^0C and 65^0C and stored at 4 ± 1^0C had keeping quality of more than three weeks against the control of two weeks (Ashok, 1990).

III. MATERIALS AND METHODS

The materials used and the methods employed in this investigation are presented in this chapter. The following studies were carried out at the Students Experimental Dairy Plant (SEDP) Dairy Science College, Karnataka Veterinary Animal and Fisheries Sciences University, Bangalore.

3.1 Materials

The following materials were used in this investigation for the preparation of enriched probiotic yoghurt.

3.1.1 Whole milk

Fresh cow milk procured from Students Experimental Dairy Plant of Dairy Science College, KVAFSU, was used for the preparation of yoghurt.

3.1.2 Skim milk and cream

Fresh skim milk and cream obtained by separating the whole milk from cow using the cream separator were used for standardizing the milk for yoghurt preparation.

3.1.3 Skim milk powder

"Nandini" brand skim milk powder manufactured by Karnataka Milk Federation (KMF) was procured from the local market to standardize the yoghurt mix.

3.1.4 Whey Protein Concentrate

Fresh spray dried Whey Protein Concentrate having 70% protein procured from Mahaan Proteins Ltd., New Delhi, was used for the enrichment of yoghurt.

3.1.5 Fruits

Fresh Mango, Sapota and Pineapple pulps were procured from the local market and used in the preparation of probiotic yoghurt.

3.1.6 Utensils and Glassware

Stainless steel vessels of varying capacities and stainless steel stirrers were used at various stages of the investigation. Conical flasks, beakers, volumetric flasks, measuring jars of Borosil brand were employed for analysis of yoghurt. Corning glass test tubes of 20ml capacity were used for propagation of starter cultures, chemical and microbiological analysis. Glasswares were cleaned by detergents and sterilized using Hot Air Oven at 160^0C – 180^0C for 2 hr and were used for microbiological analysis of the product.

3.1.7 Chemicals

Only analytical grade (AR) chemicals of highest purity were used in this investigation

3.1.8 Microbiological ingredients

Fresh ingredients for preparing various growth media to study the microbial load in the samples were used in this investigation. Ready to use medium like Rogosa SL Agar

for *Lactobacillus acidophilus* and Bifidobacterium Agar for *Bifidobacterium bifidum* were procured from HiMedia Laboratories Pvt. Ltd. Mumbai.

3.1.9 Starter cultures

The starter cultures were maintained in the Postgraduate Laboratory, Dairy Technology Department KVAFSU, Bangalore and was used in this study.

Mixed starter cultures of *Streptococcus ssp. thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus* in the ratio of 1:1 was used in the preparation of probiotic yoghurt.

Lactic cultures like *Lactobacillus acidophilus* and *Bifidobacterium bifidum* cultures procured from K.C.Das Ltd Bangalore, were used as probiotic cultures. The stock cultures were maintained in Plain Skim Milk and subcultured once in 15 days, and the working cultures are also maintained in Plain Skim Milk.

3.1.10 Packaging materials

Polystyrene cups of 100ml capacity purchased from local market were used for the judging of the product. Three different packaging materials Polypropylene (PP), polystyrene (PS) and Polyethylene teraphthalate (PET) were used for packaging of enriched probiotic yoghurt.

3.2 Equipments

Electronic Balance, Autoclave, Digital pH meter, Hot Air Oven, Incubator, Water bath, Refrigerator, Homogenizer were used in the investigation.

Sl No.	Equipment	Company
1	Homogenizer	Rannie (Copenhagen) Capacity – 100 lit/hr
2	pH meter	ELICO Pvt. Ltd.
3	Bacteriological Incubator	Serwell Instrument Inc. Bangalore
4	Water bath	Fourtech Pvt. Ltd
5	Laminar airflow chamber	Labline Instruments, Bangalore
6	Falling ball Viscometer	Gilmont Instrument Barnant Company, Barrington

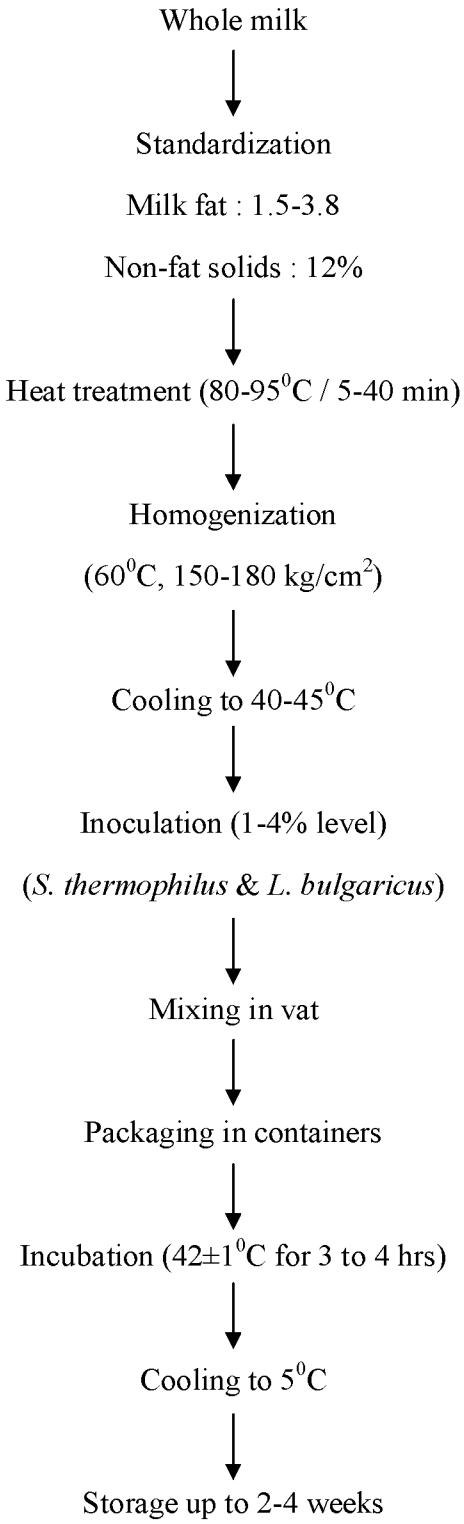
3.3 Methods

3.3.1 Experimental procedure for development of yoghurt

The procedure followed for preparation of yoghurt was adopted from Venkateshaiah (1995) with slight modifications. Fresh cow milk procured was standardized to 1.5-3.8% milk fat and 12% MSNF using skim milk powder and cream. Heat treatment was given up to 85^0C for 30 minutes followed by cooling to 43^0C. Then the milk was preheated to 60^0C -65^0C and was homogenized at 2000 psi at 1st stage and 500 psi 2nd stage and was cooled to 40-45^0C. Starter culture consisting of *Streptococcus salivarus ssp thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus* was inoculated at the rate of 1-4% and was then mixed in vat and packed in containers and incubated to 40-45^0C for 3-6 hours. After incubation it was cooled to 5^0C and stored up to 2-4 weeks.

3.3.2 Optimization of whey protein concentrates for preparation of enriched probiotic yoghurt.

Enriched yoghurt was prepared by partial replacement of MSNF with whey protein concentrate with 70% protein at three different levels like 0.5%, 1.0%, 1.5%. The resultant enriched yoghurt was subjected for various physico-chemical analyses and served to panel of judges along with control to judge the overall acceptability. Based on sensory evaluation the best combination was selected and used for further studies.



Flow chart for preparation yoghurt

3.3.3 Optimization of different fruit pulps for preparation of enriched probiotic yoghurt

Fruit pulps from mango, pineapple and sapota were added at the rate of 10%, 15% and 20% separately for every 100 gm of enriched yoghurt. Yoghurt prepared at three levels were served to panel of judges to judge its sensory characteristics. The one that secured maximum score was used for further trails.

3.3.4 Optimization of probiotic cultures for preparation of enriched probiotic yoghurt

To standardize the inoculum level in the preparation of enriched yoghurt, the mixed culture of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* were used for the preparation of yoghurt.

3.3.5 Maintenance and propagation of cultures

The stock cultures of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* were maintained in yeast glucose chalk milk of 10 ml quantities in the test tubes with cotton plug was incubated at 37^0C for 16-18 hrs. After the incubation the test tubes with good setting were stored in the refrigerator (4 ± 1^0C). These stock cultures were propagated once in a week.

3.3.6 Preparation of working culture

The working cultures were obtained from the stock cultures maintained independently. The skim milk taken in 100ml conical flasks with cotton plug was

sterilized by autoclaving at 121^{0}C for 15 min. after cooling to room temperature, 1 percent stock culture was added to the skim milk and incubated at 37^{0}C for 16 to 18 hrs.

3.3.7 Viability of the probiotic cultures in the enriched probiotic yoghurt during the storage period.

Enriched yoghurt containing mixed cultures stored at 7±1^{0}C was estimated for every week by using Rogosa SL agar media for their viability. Rogosa SL agar was used for enumerating *L. acidophilus* and in case of *B. bifidum* Bifidobacterium agar was used. Pour plate technique was adopted for the enumeration of probiotics. The samples were mixed thoroughly and decimal dilutions were made by using potassium dihydrogen phosphate (KH_2PO_4). 1 ml of the appropriate decimal dilution was transferred to sterile Petri plates in duplicate and mixed with melted and tempered (50^{0}C) media. After the agar had solidified, the plates were inverted and incubated in anaerobic jars under CO_2 atmosphere at 37^{0}C for 72 hours. The colonies developed were counted; the average counts of duplicate plates were taken and tabulated.

3.4 Analytical methods

3.4.1 Fat

Fat content in milk yoghurt was determined by Gerber method as per ISI : SP 18 (Part XI) 1981.

3.4.2 Milk Solids not Fat (MSNF)

MSNF content in milk was computed by the formula

SNF% = 0.25 CLR + 0.2 F + 0.48

Where, CLR – Corrected Lactometer Reading

F- Fat (%)

3.4.3 Total Solids (TS)

Total solids of milk, Yoghurt were determined by gravimeteric method as per ISI : SP 18(Part XI) 1981.

3.4.4 Titratable acidity of yoghurt

Yoghurt acidity was measured as per method described in ISI: SP 18 (Part XI) 1981.

3.4.5 Protein

Protein content of yoghurt was determined by Micro-Kjildhal method as per ISI: SP 18 (Part XI) 1981.

3.4.6 Moisture

Moisture was determined according to the procedure given in ISI:SP 18(Part XI) 1981.

3.4.7 Ash

The percentage of ash was determined by the procedure given under ISI:SP 18 (Part XI) 1981.

3.4.8 pH

pH of the sample was measured using a digital pH meter. (Elico Pvt. Ltd.) at 25^0C. About 20 ml representative samples were used for pH determination

3.4.9 Specific gravity

Specific gravity of samples were estimated at 20^0C by using a standard specific gravity bottle of 25ml capacity by taking distilled water as a standard liquid.

3.4.10 Viscosity

Viscosity of samples were measured using Falling Ball Viscometer at 20^0C (Gilmont instruments). The measuring tube was filled with the samples and the ball was inserted through the open end of the tube. Depending upon the falling time of the ball and the expected viscosity, different balls were used. The time taken by the ball to fall through a distance of 100 mm was measured accurately using 1/10 sec stopwatch. The viscosity was calculated by using the following formula.

$$\mu = K (pt-p) t$$

Where, μ= Viscosity in (Cp)

t = falling time of ball (minutes)

pt = Density of the ball (g/ml)

p = Density of liquid at measuring temperature (g/ml)

K = Ball constant (0.3)

Cp = centipoises

3.4.11 Syneresis

Control and heat shocked samples were stored in glass bottles (200 ml) at 7±1^{0}C. The syneresis was measured after one day and one week of storage. The amount of free whey collected was measured by pipetting using graduated 2 ml pipette. Care was taken not to disturb the product while pipeting.

3.4.12 Heating of the product

Heat treatment was given after subjecting the cold product (7±1^{0}C) for single stage homogenization (500 psi), during preliminary studies the temperatures between 55^{0}C to 65^{0}C with momentary holding were considered for heat shock. The temperatures of 55^{0}C and 65^{0}C were found to be adequate without any adverse effect on quality of the product. Continuous stirring was done during heat treatment of product and once product reached the required temperature, the steel container was removed from water bath and the product was immediately cooled to 20-25^{0}C using water and packaged in 200ml wide mouthed glass bottles and chilled to 7±1^{0}C.

3.5 Storage studies

The keeping quality of enriched probiotic yoghurt samples were tested for sixteen days at (7±1^{0}C) at one day interval and the stored samples were examined for overall acceptability by sensory evaluation and viability of probiotics by microbial analysis. The

results were tabulated and subjected for statistical analysis, and also samples were examined for overall acceptability by a panel of judges.

3.6 Sensory Analysis

The sensory analysis was carried out by serving control samples of yoghurt with experimental samples to a panel of judges with 9 point hedonic scale score card to adjudge the quality of product with respect to colour and appearance, consistency, flavour, sweetness and overall acceptability. The scores given by panel of judges were then statistically analysed. The samples were code numbered to avoid identification and bias.

3.7 Statistical analysis

The experimental results were analyzed statistically for test of significance by using ANOVA as per the procedure of Sundararaj *et al.*, (1972).

IV. EXPERIMENTAL RESULTS

Probiotic food products are catching up fast due to health consciousness among the Indian consumers. Dairy products like yoghurt, shrikhand, cheese, lassi, ice cream have been preferred probiotic carriers, as their short shelf life and refrigeration allows living organisms to remain as active cultures.

Fermented milk and milk products have become important component of diet of human beings because of their therapeutic and nutritional value. Yoghurt being one of the important fermented milk product prepared by using starter cultures. In this study an attempt has been made to enrich the yoghurt with WPC, fruit pulps from mango, sapota, pineapple and probiotic cultures like *Bifidobacterium bifidum* and *Lactobacillus acidophilus* to increase the richness of the product. Results obtained during the process of optimization, sensory attributes along with storage stability of "enriched probiotic yoghurt" with suitable tables, figures and statistical analysis are presented in this chapter.

4.1 Chemical composition of Whey Protein Concentrates (WPC) used in production of enriched probiotic yoghurt

The results of analysis of WPC for proximate composition used for manufacture of yoghurt is given in Table 1. The WPC contains 70.48% Protein, 17.80% Carbohydrates, 3.79% Fat, 3.9% Ash and 3.6% Moisture.

Process optimization for the preparation of enriched probiotic yoghurt

The results pertaining to the process optimization for the preparation of enriched probiotic yoghurt were presented in this chapter.

Table 1: Chemical composition of Whey Protein Concentrate (WPC)

Constituents	Percentage (%)
Protein	70.48
Lactose	17.80
Fat	3.79
Ash	3.90
Moisture	4.03

All the values are average of 3 trials

4.2 Effect of various yoghurt and probiotic cultures on the viability of probiotics

The effect of various starter cultures on the viability of probiotics and acidity of yoghurt are depicted in the Table 2. The maximum acidity (% LA) was obtained to yoghurt sample LS_2 (0.96) and minimum acidity was noticed in LS_3 (0.92). There was a slight difference between the acidity of control, LS_1, LS_2 and LS_3 samples.

The *S.thermophilus* log counts of control, LS_1, LS_2 and LS_3 samples were 8.67, 8.65, 8.78 and 8.87 respectively and there was a significant difference between the *S.thermophilus* log counts of control, LS_1, LS_2 and LS_3. Similarly, there was slight difference between *L.bulgaricus* log counts of control, LS_1 and LS_2 were 8.63, 8.64 and 8.71 respectively. Whereas, probiotic log count (*B.bifidum*) of LS_1 and LS_3 were 6.91 and 6.95 respectively and probiotic log count (*L.acidophilus*) of LS_2 and LS_3 were 7.20 and 7.30 respectively.

4.3 Effect of various yoghurt and probiotic cultures on the sensory characteristics of yoghurt

The control and experimental samples of probiotic yoghurt with different levels of probiotic cultures were served to panel of judges for adjudging the quality of enriched probiotic yoghurt with respect to colour and appearance, body and texture flavour and product acidity. The sensory scores obtained are presented in Table 3.

4.3.1 Colour and appearance

The sensory scores for colour and appearance of control probiotic yoghurt were 4.50 as against 4.50, 4.25 and 4.50 for probiotic yoghurt samples (LS1, LS_2 and LS_3)

Table 2: Effect of various yoghurt and probiotic cultures on the viability of probiotics

Types of cultures	Acidity (%LA)	*S.thermophilus* (log_{10} cfu/gm)	*L.bulgaricus* (log_{10} cfu/gm)	Probiotics	
				B.bifidum (log_{10} cfu/gm)	*L.acidophilus* (log_{10}cfu/gm)
Control	0.95	8.67	8.63	-	-
LS_1	0.94	8.65	8.64	6.91	-
LS2	0.96	8.78	8.71	-	7.20
LS3	0.92	8.87	-	6.95	7.30

- All the values are average of three trials.
- LS_1 : product with *L.bulgaricus*, *S.thermophilus* and *B.bifidum* (1:1:1).
- LS_2 : product with *L.bulgaricus*, *S.thermophilus* and *L.acidophilus* (1:1:1).
- LS_3 : product with *S.thermophilus*, *B.bifidum* and *L.acidophilus* (1:1:1).

Table 3: Influence of yoghurt cultures and probiotics on the sensory characteristics of yoghurt

Types of cultures	Colour and appearance (5)	Body and texture (5)	Flavour (10)	Product acidity (2)	Container & closure (3)
Control	4.50	4.50	8.50	2.0	3
LS1	4.50	4.50	8.50	2.0	3
LS_2	4.25	4.00	7.50	1.0	3
LS_3	4.50	4.50	8.50	2.0	3
CD	0.19	0.15	0.13	0.10	NS

- All the values are average of three trials.
- LS_1 : product with *L.bulgaricus*, *S.thermophilus* and *B.bifidum* (1:1:1).
- LS_2 : product with *L.bulgaricus*, *S.thermophilus* and *L.acidophilus* (1:1:1).
- LS_3 : product with *S.thermophilus*, *B.bifidum* and *L.acidophilus* (1:1:1).

respectively with different levels of probiotic cultures. It is apparent from the statistical analysis that, except LS_2, inoculation of probiotic cultures had no significant effect on colour and appearance of enriched probiotic yoghurt.

4.3.2 Flavour

The sensory scores with respect to Flavour for control sample were 8.50 as against 8.50, 7.50 and 8.50 for LS_1, LS_2 and LS_3 samples respectively. The sensory scores for Flavour did not alter and is same for control, LS_1 and LS_3 (8.50). Statistical analysis revealed that there is a significant effect on Flavour of enriched probiotic yoghurt compared to control and LS2 culture.

4.3.3 Body and Texture

The sensory scores with respect to Body and Texture for control sample were 4.50 as against 4.50, 4.00 and 4.50 for the samples LS_1, LS_2 and LS_3 respectively. The sensory scores for Body and Texture remained same for control, LS_1 and LS_3 (4.50). Sensory score is lower for the Flavour for LS_2 (4.0). Statistical analysis revealed that there is a significant effect on body and texture of enriched probiotic yoghurt compared to control and LS_2 culture.

4.3.4 Product Acidity

The sensory score for acidity of control sample is 2.0 % as against 2.0, 1.0 and 2.0 for different levels of probiotic cultures LS_1, LS_2 and LS_3 respectively. Product acidity remained same for control, LS_1 and LS_3 (2.0). The lowest product acidity score was awarded to the sample LS_2 (1.0).

4.3.4 Container and Closure

Judges awarded maximum score for all the samples.

4.4 Effect of various heat treatments of milk on the sensory characteristics of probiotic yoghurt.

The control and experimental samples were prepared by subjecting the milk to the various heat treatments *viz* pasteurization (T_1), instant boiling (T_2) and sterilization (T_3). The products were served to the panel of judges for adjudging the quality of probiotic yoghurt with respect to colour and appearance, body and texture, flavour and product acidity and the results are presented in Table 4. Sensory scores indicate that heat treatment of milk to boiling temperature (T_2) had a product with superior quality followed by pasteurization (T_1) and sterilization (T_3), whereas; sample T_1 was awarded lowest score.

The colour and appearance scores of probiotic yoghurt was maximum and similar in case of treatments T_1, T_2 and control (4.5) and lowest score was awarded to treatment T_3 (4.0). The statistical analysis revealed that there was a significant effect on colour and appearance scores of T_3 when compared to control, T_1 and T_2 samples.

The minimum body and texture score was awarded to sample T_1 (4.0) compared to T_2(4.5), T_3(4.5) and control (4.50) respectively. The statistical analysis revealed that there was a significant effect on body and texture scores of T_2, T_3 and control samples compared to T_1.

Table 4: Influence of heat treatments of milk on the sensory characteristics of probiotic yoghurt.

Types of cultures	Colour and appearance (5)	Body and texture (5)	Flavour (10)	Product acidity (2)	Container & closure (3)
Control	4.5	4.5	8.50	2.0	3
T_1	4.5	4.0	8.00	2.0	3
T_2	4.5	4.5	8.55	2.0	3
T_3	4.0	4.5	7.00	2.0	3
CD	0.18	0.15	0.20	NS	NS

All the values are average of three trials.

T_1 : Heated to Pasteurization temperature.

T_2 : Heated to instant boiling temperature.

T_3 : Heated to Sterilization temperature.

The maximum Flavour score was awarded to sample T_2 (8.55), followed by control (8.5) and the lowest score was awarded to T_3 (7.00). Whereas, the T_1 (pasteurization) was awarded a Flavour score of 8.0. The statistical analysis revealed that effect of heating temperature had significant effect on Flavour of probiotic yoghurt .

From the results presented in Table 4, it is apparent that the product acidity scores for probiotic yoghurt did not alter (2.0) for samples T_1, T_2, T_3 and control samples. There is no significant effect on the product acidity scores of T_1, T_2, T_3 and control samples.

4.5. Effect of various heat treatments of milk on the viability of probiotics and acidity of probiotic yoghurt.

The effect of various heat treatments of milk on the viability of probiotics and acidity of enriched probiotic yoghurt is presented in Table 5. The acidity (%LA) of experimental samples of T_1, T_2 and T_3 were 0.91, 0.94 and 0.92 % lactic acid respectively. There was no significant difference between the acidity among experimental samples of T_1, T_2 and T_3.

The various heat treatments of milk influenced the growth of *B.bifidum* and *L.acidophilus*. The viability count was significantly higher when the milk was heated to sterilization temperature (7.35 $\log_{10}$ cfu/gm for *B.bifidum* and 7.44 $\log_{10}$ cfu/ml for *L.acidophilus*). The viability count was lower in milk heated to pasteurization temperature (6.20 $\log_{10}$ cfu/ml for *B.bifidum* and 6.50 $\log_{10}$ cfu/gm for *L.acidophilus*) compared to viable counts of T_2 and T_3.

Table 5: Effect of various heat treatments of milk on the viability of probiotics and acidity of probiotic yoghurt.

Types of heat treatments	Viable counts (Log_{10} Cfu/Gm)		Acidity % Lactic acid
	B.bifidum	***L.acidophilus***	
T_1	6.20	6.50	0.91
T_2	7.17	7.32	0.94
T_3	7.35	7.44	0.92

- All the values are average of three trials.
- T_1 : Heated to Pasteurization temperature.
- T_2 : Heated to instantaneous Boiling temperature.
- T_3 : Heated to Sterilization temperature.

4.6. Effect of different levels of WPC on the sensory characteristics of enriched probiotic yoghurt.

The effect of addition of different levels of WPC at 0.5, 1 and 1.5% on the sensory characteristics of enriched probiotic yoghurt is presented in Table 6.

The colour and appearance scores of enriched probiotic yoghurt samples were,(4.50) control, (4.50) 0.5%, (4.5), 1.0% (4.65) and 1.5% (4.50). The statistical analysis revealed that there was no significant effect on the colour and appearance scores of control and enriched probiotic yoghurt samples.

The maximum body and texture score was awarded to 1.0% (4.77) compared to 0.5% (4.50), control (4.50) and the lowest body and texture score was awarded to 1.5% (4.21). The statistical analysis revealed that there was a significant effect on the body and texture scores of control and enriched probiotic yoghurt samples.

The maximum Flavour score was awarded to 0.5% (8.50) and control (8.50), followed by 1.0% (8.47) and the lowest Flavour score was awarded to 1.5% (7.30). The statistical analysis revealed that the effect of addition of WPC had significant effect on Flavour scores of probiotic yoghurt samples.

From, the results presented in Table 6, it is apparent that the acidity scores for enriched probiotic yoghurt remained same (1.50) for samples 0.5%, 1% and control samples. The lowest score was awarded to 1.5% (1.0). There is a significant effect on the product acidity scores of 1.5% with control and enriched probiotic yoghurt samples. The scores awarded for container and closure remains maximum for all the samples.

Table 6: Effect of different levels of WPC on the sensory characteristics of probiotic yoghurt

Level (%) WPC	Colour and appearance (5)	Body and texture (5)	Flavour (10)	Product acidity (2)	Container & closure (3)
Control	4.50	4.50	8.50	1.50	3
0.5%	4.50	4.50	8.50	1.50	3
1.0%	4.65	4.77	8.47	1.50	3
1.5%	4.50	4.21	7.30	1.0	3
CD	NS	0.25	0.53	0.11	NS

All the values are average of three trials.

4.7. Effect of incorporation of WPC on the chemical composition and acidity of probiotic yoghurt

The effect of incorporation of WPC at 0.5, 1 and 1.5% level on the chemical composition and acidity of enriched probiotic yoghurt is presented in Table 7.

The maximum fat percentage is seen in case of control (4.5%), followed by 0.5% WPC level (4.47), 1.0% (4.45) and minimum fat is seen in 1.5% added WPC sample which is about 4.42. The statistical analysis revealed that there was no significant effect on the fat percentage of control and enriched probiotic yoghurt samples.

The protein content of 0.5%, 1%, 1.5% WPC incorporated samples and control samples were 3.76, 4.25, 4.58 and 3.60% respectively. Maximum protein content is found in 1.5% WPC incorporation sample (4.58), compared to 0.5% (3.76), 1% (4.25) and the lowest protein found in control (3.60). The statistical analysis revealed that there was a significant effect on the protein content of control and enriched probiotic yoghurt samples.

From the results presented in Table 6, it is apparent that the product acidity scores for enriched probiotic yoghurt remained same (0.75) for samples 0.5% WPC and control samples, followed by 0.74 for 1.0% WPC. The lowest was found in 1.5% level (0.71). There was no significant effect on the product acidity of control and enriched probiotic yoghurt samples.

Table 7: Effect of incorporation of WPC on the chemical composition and acidity of probiotic yoghurt

Level of WPC %	Fat	Protein	Product Acidity (% LA)
Control	4.50	3.60	0.75
0.5	4.47	3.76	0.75
1.0	4.45	4.25	0.74
1.5	4.42	4.58	0.71
CD	0.06	0.23	0.17

All the values are average of three trials.

4.8. Effect incorporation of WPC on the viability of probiotics and acidity of enriched probiotic yoghurt

The effect of addition of different levels of WPC at 0.5, 1.0 and 1.5% level on the viability of probiotics and acidity of enriched probiotic yoghurt is presented in Table 8. The acidity (%LA) in control and enriched probiotic yoghurt samples with WPC at 0.5, 1 and 1.5% levels were 0.75, 0.76, 0.77 and 0.78 % respectively. There was slight difference between the acidity of control and treated samples.

Addition of different levels of WPC did influence the growth of *B.bifidum* and *L.acidophilus* as it was added as probiotic substance. The viable count was higher in 1.5% WPC (7.86 $\log_{10}$ cfu/gm for *L.acidophilus)* and higher in 1% (7.55 $\log_{10}$ cfu/gm for *B.bifidum*). The viable count was lower in 0.5% WPC (7.63 $\log_{10}$ cfu/gm for *L.acidophilus,* 7.45 $\log_{10}$ cfu/gm for *B.bifidum*). There was a significant difference between the viable log counts of control and enriched probiotic yoghurt samples.

4.9. Effect of different levels of mango pulp on the sensory characteristics of enriched probiotic yoghurt

The effect of addition of different levels of mango pulp (10, 15 and 20% level) on the sensory characteristics of enriched probiotic yoghurt is presented in Table 9.

The colour and appearance scores of enriched probiotic yoghurt samples with mango pulp were 4.5, 4.75, 4.5 for 10%, 15%, 20% level of incorporation and control secured score of 4.5 out of 5. The statistical analysis revealed that there was a significant effect on the colour and appearance scores of control and 15% sample.

Table 8: Effect of WPC on the viability of probiotic and acidity of probiotic yoghurt

Level of WPC (%)	*L.acidophilus* (log_{10} cfu/gm)	*B.bifidum* (log_{10} cfu/gm)	Acidity % LA
Control (0)	7.45	6.95	0.75
0.5	7.63	7,45	0.76
1.0	7.81	7.55	0.77
1.5	7.86	7.51	0.78

All the values are average of three trials.

Table 9: Effect of different levels of mango pulp on the sensory characteristics of enriched probiotic yoghurt.

Levels of mango pulp (%)	Colour & appearance (5)	Body & texture (5)	Flavour (10)	Product acidity (2)	Container & closure (3)
Control	4.5	4.50	8.50	1.5	3
10	4.5	4.50	8.65	1.5	3
15	4.75	4.75	8.75	1.5	3
20	4.5	4.00	8.50	1.25	3
CD	0.18	0.11	0.20	0.10	NS

All the values are average of three trials.

The maximum body and texture score was awarded to 15% level (4.75) compared to 10% level (4.50), control (4.50) and the lowest body and texture score was awarded to 20% level (4.0). Statistical analysis revealed that there was a significant effect on the body and texture scores control and enriched probiotic yoghurt samples.

The highest Flavour score was awarded to 15% level (8.75), followed by 10% (8.65), whereas the control (8.50) and 20% level (8.50) had the lowest score. The statistical analysis revealed that the effect of addition of different levels of mango pulp had significant effect on Flavour scores of yoghurt samples.

From the results presented in Table 9, it is apparent that the product acidity scores for enriched probiotic yoghurt with different levels of addition of mango pulp were control (1.5), 10% level (1.5), 15% level (1.5) and 20% level (1.25). The lowest score was awarded to 20% level (1.25). The statistical analysis revealed that the effect of addition of different levels of mango pulp was significant on product acidity scores of yoghurt samples. Whereas, container and closure scores remained maximum for all the samples.

4.10. Effect of different levels of Sapota pulp on the sensory characteristics of enriched probiotic yoghurt.

The effect of addition of different levels of Sapota pulp at 10, 15 and 20% level on the sensory characteristics of enriched probiotic yoghurt is presented in Table 10.

The colour and appearance scores of enriched probiotic yoghurt samples with Sapota pulp were 10% (4.50), 15% (4.75), 20% (4.50) and control (4.50). The statistical

analysis revealed that there was a significant effect on the colour and appearance scores of control and 15% sample.

The maximum body and texture score was awarded to 15% level (4.80) compared to 10% level (4.50), control (4.50) and the lowest body and texture score was awarded to 20% level (4.0). Statistical analysis revealed that there was a significant effect on the body and texture scores of control and enriched probiotic yoghurt samples.

The highest Flavour score was awarded to 15% level (8.75), followed by 10% (8.55), control (8.50) and 20% level (8.20) had the lowest score. The statistical analysis revealed that the effect of addition of different levels of sapota pulp was significant on Flavour of yoghurt samples.

From the results presented in Table 10, it is apparent that the product acidity scores for enriched probiotic yoghurt with different levels of addition of sapota pulp were control (1.5), 10% level (1.5), 15% level (1.5) and 20% level (1.0). The lowest score was awarded to 20% level (1.0). The statistical analysis revealed that the effect of addition of different levels of sapota pulp was significant on product acidity of yoghurt samples. Whereas, container and closure scores remained maximum for all the samples.

4.11. Effect of different levels of pineapple pulp on the sensory characteristics of enriched probiotic yoghurt.

The effect of addition of different levels of pineapple pulp (10, 15 and 20% level) on the sensory characteristics of enriched probiotic yoghurt is presented in Table 11.

Table 10: Effect of different levels of Sapota pulp on the sensory characteristics of enriched probiotic yoghurt.

Levels of sapota pulp (%)	Colour & appearance (5)	Body & texture (5)	Flavour (10)	Product acidity (2)	Container & closure (3)
Control	4.5	4.50	8.50	1.5	3
10	4.5	4.50	8.55	1.5	3
15	4.75	4.80	8.75	1.5	3
20	4.5	4.00	8.20	1.0	3
CD	0.12	0.23	0.18	0.10	NS

All the values are average of three trials.

The colour and appearance scores of enriched probiotic yoghurt samples with pineapple pulp were 10% (4.50), 15% (4.75), 20% (4.50) and control (4.50). The statistical analysis revealed that there was a significant effect on the colour and appearance scores of control and 15% sample.

The maximum body and texture score was awarded to 15% level (4.75) compared to 10% level (4.50), control (4.50) and the lowest body and texture score was awarded to 20% level (3.5). Statistical analysis revealed that there was a significant effect on the body and texture score of control and enriched probiotic yoghurt samples.

The highest Flavour score was awarded to 15% level (8.75), followed by 10% (8.55), control (8.50) and 20% level (8.25) had the lowest score. The statistical analysis revealed that the effect of addition of different levels of pineapple pulp was significant on Flavour of yoghurt samples.

From the results presented in Table 11, it is apparent that the product acidity scores for enriched probiotic yoghurt with different levels of addition of pineapple pulp were control (1.5), 10% level (1.5), 15% level (1.5) and 20% level (1.0). The lowest score was awarded to 20% level (1.0). The statistical analysis revealed that the effect of addition of different levels of pineapple pulp was significant on product acidity of yoghurt samples. Whereas, container and closure scores remained maximum for all the samples.

Table 11: Effect of different levels of pineapple pulp on the sensory characteristics of enriched probiotic yoghurt.

Pineapple pulp (%)	Colour & appearance (5)	Body & texture (5)	Flavour (10)	Product acidity (2)	Container & closure (3)
Control	4.5	4.50	8.50	1.5	3
10%	4.5	4.50	8.55	1.5	3
15%	4.75	4.75	8.75	1.5	3
20%	4.5	3.50	8.25	1.0	3
CD	0.14	0.23	0.18	0.11	NS

All the values are average of three trials.

4.12. Effect of addition of various fruit pulps on the sensory characteristics of enriched probiotic yoghurt

The results pertaining to the addition of various fruit pulps on colour and appearance, body and texture, flavour, and acidity of enriched probiotic yoghurt are presented in Table 12.

4.12.1. Colour and appearance

The maximum colour and appearance score was awarded to pineapple at 15% level (4.75), followed by mango at 15% level (4.50), control (4.50) and the lowest score was awarded to sapota at 15% level (4.0) The statistical analysis confirmed that, there is a significant effect on the colour and appearance of enriched probiotic yoghurt with incorporation of fruit pulps like mango, pineapple and sapota.

4.12.2. Body and texture

The body and texture scores of enriched probiotic yoghurt samples with mango pulp, pineapple pulp and sapota pulp at 15% level were 4.50, 4.75, 4.50 respectively, and control (4.50). The statistical analysis revealed that there was a significant effect on the body and texture scores of control and 15% sample.

4.12.3. Flavour

The highest flavour score was awarded to yoghurt enriched with pineapple at 15% level (9.25), followed by mango pulp at 15% level (8.75), control (8.50) and the lowest score was awarded to sapota pulp at 15% level (8.0). The statistical analysis revealed that the effect of addition of different levels of fruit pulps was significant on Flavour of yoghurt samples.

Table 12 : Effect of various fruit pulps on the sensory characteristics of enriched probiotic yoghurt

Sample	Colour & appearance (5)	Body & texture (5)	Flavour (10)	Product acidity (2)	Container & closure (3)
Control	4.5	4.50	8.50	1.5	3
Mango	4.5	4.50	8.75	1.5	3
Pineapple	4.75	4.75	9.25	1.5	3
Sapota	4.0	4.50	8.0	1.5	3
CD	0.13	0.16	0.22	NS	NS

- All the values are average of three trials.
- Mango pulp
- Pineapple
- Sapota

} Used at 15% level of concentration.

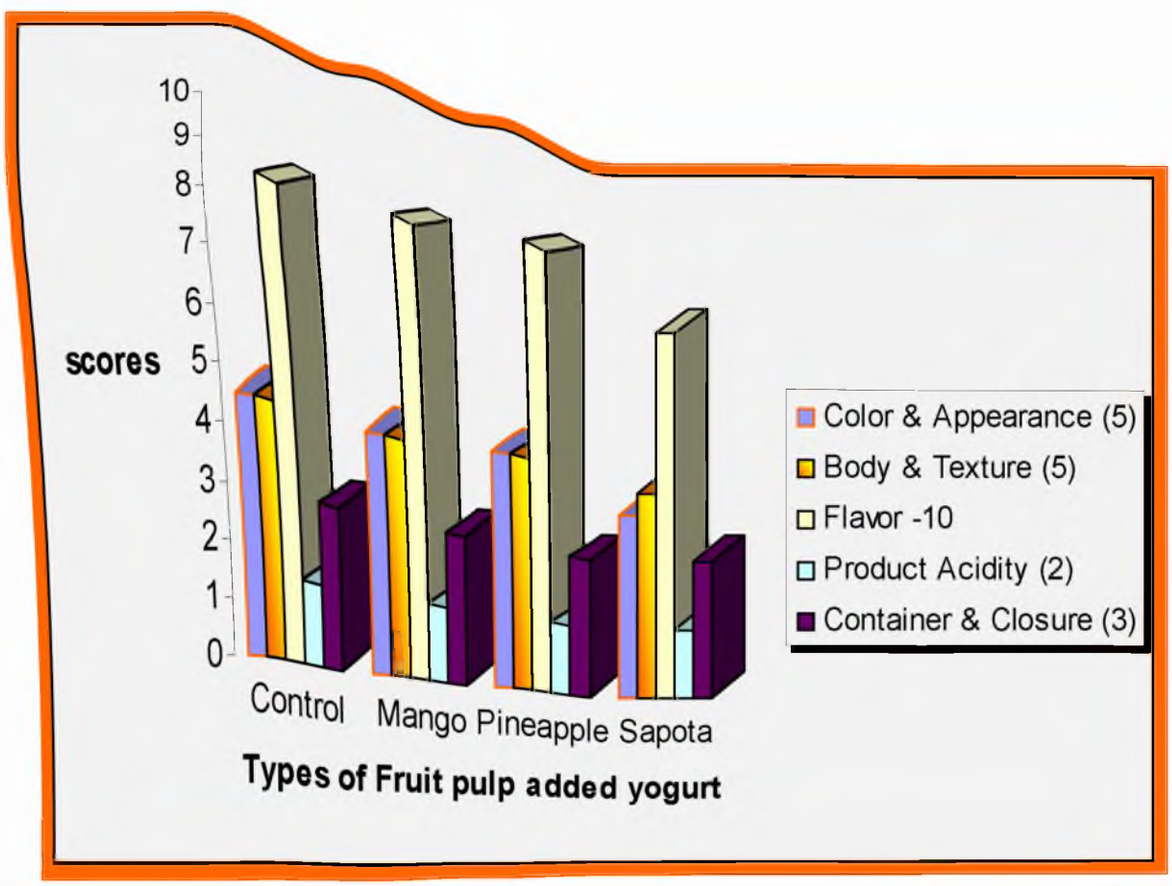


Fig. 1 : Effect of various fruit pulps on the sensory characteristics of enriched probiotic yoghurt.

4.12.4. Product acidity

From the results presented in Table 12, it is apparent that the product acidity scores for enriched probiotic yoghurt with different levels of addition of fruit pulps were control (1.5), mango pulp at 15% level (1.5), pineapple at 15% level (1.5) and sapota pulp at 15% level (1.5). The statistical analysis revealed that the effect of addition of different levels of fruit pulps has no significant effect on product acidity of yoghurt samples.

4.13. Effect of packaging material on the acidity of control and enriched probiotic yoghurt stored at 7±1^{0}C

The effect of packaging materials on the acidity of control and enriched probiotic yoghurt stored at 7±1^{0}C temperature is presented in Table 13.

The acidity of fresh control yoghurt sample packed in PP, PS and PET remained same (0.85% LA). The acidity of yoghurt packed in PP, PS and PET on second day of storage was 0.93, 0.94 and 0.87% LA respectively and there was no significant difference between the acidity of samples packed in different packaging material. On 4th day of storage, the acidity of samples packed in PP,PS and PET were 1.09, 1.10 and 0.93 %LA respectively and there was no significant difference between the sample in 3 different packaging materials. On 6th day of storage, the acidity of samples packed in PP,PS and PET were 1.32, 1.36 and 0.98 %LA respectively.

On 8th day of storage, the acidity of samples packed in PP, PS and PET were 1.56, 1.61 and 1.13 %LA respectively and there was no significant difference the different packaging materials. On 10th day of storage, the acidity of samples packed in PET was 1.54 %LA.

Table 13 : Effect of packaging materials on the acidity (%LA) of control and enriched probiotic yogurt stored at 7±1^{0}C

Days		0	2	4	6	8	10	12	14	16	18
Control	PP	0.85	0.93	1.09	1.32 spoiled	-	-				
	PS	0.85	0.94	1.10	1.36 spoiled	-	-				
	PET	0.85	0.87	0.93	0.98	1.13	1.54 spoiled				
Enriched probiotic yoghurt	PP	0.82	0.90	0.95	1.07	1.11	1.19	1.22 spoiled		-	
	PS	0.82	0.91	0.98	1.09	1.17	1.22	1.26 spoiled		-	-
	PET	0.82	0.80	0.85	0.93	0.98	1.00	1.03	1.18	1.26	1.58 spoiled

The acidity of fresh enriched probiotic yoghurt sample packed in PP, PS and PET remained same (0.82% LA). The acidity of yoghurt packed in PP, PS and PET on second day of storage were 0.90, 0.91 and 0.80% LA respectively and there was not much difference between the acidity of samples packed in different packaging material. On 4^{th} day of storage, the acidity of samples packed in PP, PS and PET were 0.95, 0.98 and 0.85 %LA respectively and there was not much difference between the samples packed in different packaging materials. On 6^{th} of day storage, the acidity of samples packed in PP,PS and PET were 1.07, 1.09 and 0.93 %LA respectively

On 8^{th} of day of storage, the acidity of samples packed in PP, PS and PET were 1.11, 1.17 and 0.98 %LA respectively and the difference between the acidity of product was found to be not significant. On 10^{th} day of storage, the acidity of samples packed in PP, PS and PET were 1.19, 1.22 and 1.00 %LA respectively and there was no significant difference the different packaging materials. On 12^{th} day of storage, the acidity of samples packed in PP,PS and PET were 1.22, 1.26 and 1.03 %LA respectively and there was not much difference between the different packaging materials. On 14^{th} day of storage, the acidity of samples packed in PET was 1.18 %LA. Similarly on 16^{th} day 1.26 %LA followed by 1.58 %LA on 18^{th} day.

4.14. Effect of packaging material on the viability (log_{10} cfu/gm) of probiotics of enriched probiotic yoghurt stored at 7 ± 1^0C

The viability of probiotics in the enriched probiotic yoghurt packed on three different packaging materials *viz* PP, PS and PET stored at 7 ± 1^0C is presented in Table 14.

The results indicate that there was a slight decrease in the viability of *B.bifidum* and *L.acidophilus* counts in the enriched probiotic yoghurt (PP). The *B.bifidum* counts decreased from 7.05 to 6.65 $\log_{10}$ cfu/gm and *L.acidophilus* counts decreased from 7.90 to 6.50 $\log_{10}$ cfu/gm respectively. Similarly there was a slight decrease in viability of probiotic cultures in enriched probiotic yoghurt (PS). The *B.bifidum* counts decreased from 7.05 to 6.20 $\log_{10}$ cfu/gm and *L.acidophilus* counts decreased from 7.90 to 6.10 $\log_{10}$ cfu/gm respectively. There was a slight decrease in viability of probiotic cultures in enriched probiotic yoghurt (PET). The *B.bifidum* counts decreased from 7.05 to 6.20 $\log_{10}$ cfu/gm and *L.acidophilus* counts decreased from 7.90 to 6.10 $\log_{10}$ cfu/gm respectively.

Table 14 : Effect of packaging materials on the viability of probiotics (log cfu/gm) in enriched probiotic yoghurt stored at $7\pm1^{0}C$

Storage Days	PP			PS			PET		
	St	Bb	La	St	Bb	La	St	Bb	La
0	8.90	7.05	7.90	8.90	7.05	7.90	8.90	7.05	7.90
5	8.60	7.00	7.30	8.50	6.80	7.20	8.30	6.40	6.50
10	8.00	6.65	6.50	7.30	6.20	6.40	7.30	6.50	6.30
15	- Spoiled -			- Spoiled -			6.80	6.20	6.10
20							- Spoiled -		

V. DISCUSSION

Yoghurt is a fermented mik product produced from the usual yoghurt starter cultures of *Sterptococcus salivarius ssp. thermophilus and Lactobacillus delbrueckii ssp. Bulgaricus*. Over th e years, research into yoghurt processes and ingredients has led to improvement in product quality and consistency. Fermented milks are popular in view of their organoleptic and other properties such as characteristic flavour, refreshing taste and improved digestibility. The composition of fermented milk can be easily tailored to meet various dietary requirements

In this chapter, the results pertaining to process optimization for developing enriched probiotic yoghurt by incorporating WPC, fruit pulps, probiotic cultures and their influence on various physico-chemical, sensory attributes, microbiological aspects and storage studies were discussed.

5.1 Influence of yoghurt and probiotic cultures on the viability of probiotics of yoghurt

The effect of different levels of probiotic cultures on the viability counts and acidity in probiotic yoghurt are prescribed in Table-2.

It was observed that the acidity (% lactic acid) of sample LS3 was lower (0.92) compared to the acidity of LS1 (0.94), LS2 (0.96) and control (0.95). The lower acidity of LS3 sample might be due to the slow growing cultures such as *S .thermophilus* and *B .bifidum* and whereas, *L .bulgaricus* produces acidity even stored at refrigeration temperature. Therefore LS3 combination showed a increase in viability of probiotics

counts of 6.95 and 7.30 (log cfu/ml) for *B. bifidum* and *L. acidophilus* respectively. Dave and Shah (1998) also reported that the presence of *L. delbruki ssp bulgaricus* in yoghurt leads to a higher lactic acid production during storage at refrigeration temperature.

5.2 Effect of yoghurt and probiotics on the sensory characteristics of yoghurt

Yoghurt was prepared using cultures along with probiotic cultures having different combinations

a. *S.thermophilus* + *L.bulgaricus* + *B.bifidum* (LS1)

b. *S.thermophilus* + *L.bulgaricus* + *L.acidophilus* (LS2)

c. *S.thermophilus* + *B.bifidum* + *L.acidophilus* (LS3)

The effect of various starter cultures along with probiotics on the sensory characteristics of yoghurt was presented in Table 3. The organoleptic quality of yoghurt prepared by using LS3 combination was significantly superior compared to other samples. LS2 sample has less colour and appearance scores compared to other samples (LS1, LS3 and control), LS2 sample had poor body and texture (has got a score of 4.0 out of 5.0) because of loose body and grainy texture and also due to high acidity (Table 2). Yoghurt with *S.thermophilus* + *B.bifidum* + *L.acidophilus* showed significantly acidic taste.

The yoghurt with LS3 combination of cultures showed a pleasant flavour, improved body and texture and highly appealing colour and appearance as indicated by the higher organoleptic scores. The overall acceptability of yoghurt prepared using LS3 combination (*S.thermophilus* + *B.bifidum* + *L.acidophilus)* was significantly superior

compared to other combinations (LS1 and LS2) of cultures as it yielded pleasant flavour, firm body and texture. Therefore LS3 combination was used for the further studies.

On contrary Suryawanshi *et al.*,(1993) reported inoculated *L.acidophilus* for possible therapeutic benefits with *S.thermophilus*, *L.casei* and *Lactococcus lactis biovar diacetyllactis* to impart superior flavour, taste and body and texture characteristics to the product obtained with *S.thermophilus* as compared with *L.acidophilus*, *S.lactis* and *S.cremoris*.

5.3. Effect of various heat treatments of milk on the sensory characteristics of probiotic yoghurt

The primary objective of heat treatment of milk is to destroy heat labile inhibitors as well as the competing microorganisms (pathogens) residing in the raw milk

Effect of heating temperature of milk was studied at 85°C/30 minutes (Control), Pasteurization (T1), Boiling (T2) and Sterilization temperatures (T3) (Table 4). The color and appearance scores of probiotic yoghurt was maximum in case of treatments T1, T2 and control (4.5) respectively and lowest score was awarded to treatment T3 (4.0).

The minimum body and texture score was awarded to sample T1 (4.0) compared to T2, T3 and control (4.50 respectively). The poor body and texture of sample (T1) may be due to soft curd formation and it may be because of insufficient heat treatment, which leads to less amount of whey protein denaturation caused to weaker coagulum in pasteurized milk compared to the other heating temperature.

The maximum flavor score was awarded to sample T2 (8.55), followed by control (8.5) and the lowest score was awarded to T3 (7.00), whereas the T1 (pasteurization) was awarded a flavor score of 8.0. Whereas T3 sample reported poor flavour score (7.0 out of 10.0), and this may be due to presence of pronounced cooked or heated flavour in the final product. T1 sample has poor flavour compared to T2 and T3 due to flat taste of the product. From the results presented in table 4, it is apparent that the product acidity scores for enriched probiotic yoghurt were remained same (2.0) for samples T1, T2, T3 and control samples.

5.4. Effect of various heat treatments of milk on the viability of probiotics and acidity of probiotic yoghurt

The various heat treatments of milk did influence the growth of *B.bifidum* and *L.acidophilus*. The viable log counts were higher when the milk heated at sterilization temperature (7.35 $\log_{10}$ cfu/gm for *B.bifidum* and 7.44 $\log_{10}$ cfu/gm for *L.acidophilus*). This may be due to the denaturation of whey proteins which releases the sulphhydryl compounds which stimulate the growth of probiotics. This was similar with the findings of Webb *et al.*, (1973) who reported that heating of milk to sterilization temperature is optimum for better culture growth and denaturation of whey proteins.

Deliaglio (1988) also reported that heating of milk resulted in protein alterations, leading to higher availability of peptides, amino acids and stimulatory sulphydryl compounds for the better growth of starter cultures.

5.5. Effect of various levels of WPC on the sensory characteristics of enriched probiotic yoghurt

Because of unique inherent functional and nutritional properties, WPC could be well utilized in various dairy products formulations to enhance consumer's acceptability by improving the functional, nutritional and therapeutic value of the products (Morr and Foegeding, 1990).

The effect of addition of WPC at 0.5, 1 and 1.5% level on the sensory characteristics of enriched probiotic yoghurt is presented in Table 6. The colour and appearance scores awarded for enriched probiotic yoghurt samples was 4.50 (control), 4.50 (0.5%), 4.65 (1%) and 4.50 (1.5%).The maximum body and texture score was awarded to 1% WPC (4.77) compared to 0.5% WPC (4.50), control (4.50) and the lowest body and texture score was awarded to 1.5% (4.21).There is no significance effect on colour and appearance scores of control and WPC samples. The sample containing 1.5% WPC showed poor body and texture score may be due to heavy body and also posses poor flavour scores. Further the product had acidic flavour because WPC act as prebiotics and added WPC also contributes sustainable amount of lactic acid due to the presence of lactose in WPC (Table 6).

Various authors also reported the effect of WPC in product development (Venkateshaiah, 1995; Jayaprakasha, 1998; Arunkumar, 2006). Incorporation of WPC at 5 % level in khoa showed improved sensory attributes over the control khoa, which was prepared from cow milk (Patel *et al.*, 1993). Blending of WPC at 5 % level in the preparation of rosogolla without affecting any physico-chemical and sensory attributes

was reported by Suma (2002). Use of whey protein concentrate (WPC) from 3 to 10 % in the manufacture of processed cheese gave a softer consistency, elasticity and viscosity (Kerry, 1979) and also a reduction in brittleness, improved water binding properties, improved mouth feel and spreadability (Gupta and Thapa, 1991). Yoghurt with WPC upto 20 % was found to be firmer with less synersis (Jelen *et al.*, 1987). The yoghurt was found to have less curd tension with increase in WPC (Mohammed and Abd-El-salam, 1991).

5.6. Effect of incorporation of WPC on the chemical composition and acidity of probiotic yoghurt

The effect of incorporation of different levels of WPC (0.5, 1 and 1.5%) on the chemical composition and acidity of enriched probiotic yoghurt is presented in Table 7.

It is seen from the Table 7 that the fat percentage of 4.5, 4.47, 4.45 and 4.42 was obtained with control, 0.5%, 1.0% and 1.5% of WPC respectively. The statistical analysis revealed that there was no significant difference on the fat percentage of control and enriched probiotic yoghurt samples except at 1.5% WPC level.

The protein content of 0.5%, 1%, 1.5% with WPC and control samples were 3.76, 4.25, 4.58 and 3.60% respectively. Maximum protein content was found in 1.5% (4.58), compared to 0.5% (3.76), 1% (4.25) and the lowest protein was found in control (3.60). Sample having 1.0% WPC contains significantly higher percentage of protein (4.25) compared to control, whereas no significant effect on fat (4.45) content. But the sample having 1.5% WPC contains significantly higher percentage of protein (4.58%) and total

solids (87.15), but significantly less fat (4.42) compared to control. Therefore this sample had poor flavour and heavy body.

From the results presented in Table 7, it is apparent that the product acidity scores for enriched probiotic yoghurt were same (0.75 %LA) for samples 0.5% and control samples, followed by 0.74 %LA for 1.0%. The lowest was found in 1.5% (0.71 %LA). There is no significant effect on the product acidity of 1.5% with control and enriched probiotic yoghurt samples.

5.7. Effect of incorporation of WPC on the viability of probiotics and acidity of enriched probiotic yoghurt

The effect of incorporation of different levels of WPC (0.5, 1.0 and 1.5%) on the viability of probiotics and acidity of enriched probiotic yoghurt is presented in Table 8. The acidity (%LA) in control and enriched probiotic yoghurt samples with WPC at 0.5, 1 and 1.5% levels were 0.75, 0.76, 0.77 and 0.78% of lactic acid respectively. The increase in the acidity of WPC added samples was may be due to probiotic effect of WPC.

At higher levels of WPC incorporation, increase in the counts (cfu/ml) was observed. This may be because of the added WPC, which acts as prebiotic. This was similar with the findings of Dave and Shah (1998) who reported that the addition of 2 to 5 % of whey protein concentrate (WPC) improved the viability of probiotic (*Bifidobacteria*) to a variable extent.

5.8. Effect of different levels of mango pulp on the sensory characteristics of enriched probiotic yoghurt

With the growing interest in the diversification of food products to attract consumers, in the recent past many attempts have been made to incorporate different additives into yoghurt to improve functional and nutritional properties (Bardale *et al.*,1986).

The effect of addition of different levels of mango pulp at 10,15 and 20% level, on the sensory characteristics of enriched probiotic yoghurt is presented in Table 9.

The colour and appearance scores of enriched probiotic yoghurt sample with mango pulp at 10% level secured 4.5 out of 5. The mango pulp incorporated at 15% level in yoghurt obtained sensory score of 4.75 out of 5, which is slightly higher than 10%level (4.5 out of 5). Similarly yoghurt added with mango pulp at 20% level has secured sensory score of 4.5 out 5.

The maximum body and texture score of 4.75 was awarded to yoghurt containing mango pulp at 15% level. Similarly scores of 4.5 was obtained for both control and yoghurt sample containing 10% level of mango pulp. The lowest body and texture score of 4.0 out of 5 was awarded to sample with 20% level of mango pulp. Statistical analysis reveled that there was a significant difference on the body and texture scores of control and enriched probiotic yoghurt sample with 15% mango pulp.

The highest sensory score for flavour, 8.75 out of 10 was awarded to yoghurt sample containing 15% level of mango pulp. The score of 8.65 out of 10 obtained for

sample at 10% level. The control sample and yoghurt added with 20% level mango pulp secured same sensory scores 8.5 out of 10.The statistical analysis reveled that the effect of addition of different levels of mango pulp had significant effect on flavour scores of yoghurt samples with 15% mango pulp.

From the results presented in table 9, it is apparent that the product acidity scores for enriched probiotic yoghurt remains to be same that is 1.5 out of 2 for control, sample with 10% and sample with 15% level of mango pulp incorporation. The lowest sensory score was awarded to sample containing 20% level, which secured a least sensory score of 1.25 out of 2.The statistical analysis reveled that the effect of addition of different levels of mango pulp was significant on product acidity of yoghurt samples.

5.9. Effect of different levels of Sapota pulp on the sensory characteristics of enriched probiotic yoghurt

The effect of addition of different levels of sapota pulp at 10, 15, and 20% level, on the sensory characteristics of enriched probiotic yoghurt is presented in Table 10.

The color and appearance scores of enriched probiotic sample with sapota pulp at 10% level, 20% level and control sample has secured sensory score of 4.5 out of 5. The highest LA sensory score was awarded to sample containing 15% level of sapota pulp obtained score of 4.75 out 5. The statistical analysis reveled that there was a significant effect on the color and appearance scores of control and sample with 15% sapota pulp.

The maximum body and textured score of 4.8 out of 5 was awarded to yoghurt containing sapota pulp at 15% level. Similarly control and sample with 10% level of

sapota pulp secured same score 4.5 out of 5.The lowest body and texture score, 4.0 out of 5 was awarded to sample with 20% level of sapota pulp. Statistical analysis reveled that there was a significant effect on body and texture scores of control and enriched probiotic yoghurt samples.

The highest flavour score of 8.75 out of 10 was awarded to yoghurt sample containing 15% level of sapota pulp and similarly sensory score of 8.55out of 10 was awarded to sample with 10% level, followed sensory score of 8.50 for control sample. The lowest sensory score was awarded to sample containing 20% level of sapota pulp (8.20). The statistical analysis revealed that the effect of addition of different levels of sapota pulp was significant on flavour of yoghurt samples.

From the results presented in Table 10, it is apparent that the product acidity scores of enriched probiotic yoghurt remains same (1.5 out of 2) for control, sample with 10% and sample with 15% level of sapota pulp incorporation. The lowest acidity score was awarded to sample containing 20% level, which secured a least sensory score of 1.0 out of 2. The statistical analysis revealed that the effect of addition of different levels of sapota pulp was significant on product acidity of yoghurt samples.

5.10. Effect of different levels of pineapple pulp on the sensory characteristics of enriched probiotic yoghurt

The effect of addition of different levels of sapota pulp at 10, 15, and 20% level, on the sensory characteristics of enriched probiotic yoghurt is presented in table 11.

The color and appearance scores of enriched probiotic sample with pineapple pulp at 10% level, 20% level and control sample has secured sensory score of 4.5 out of 5. The highest sensory score was awarded to sample containing 15% level of pineapple pulp obtained score of 4.75 out 5. The statistical analysis reveled that there was a significant effect on the color and appearance scores of control and 15% sample.

The maximum body and texture score of 4.75 out of 5 was awarded to yoghurt containing pineapple pulp at 15% level. Similarly control and sample with 10% level of pineapple pulp secured same score 4.5 out of 5.The lowest body and texture score, 3.5 out of 5 was awarded to sample with 20% level of pineapple pulp. Statistical analysis reveled that there was a significant effect on body and texture scores of control and enriched probiotic yoghurt samples.

The highest flavour score of 8.75 out of 10 was awarded to yoghurt sample containing 15% level of pineapple pulp and similarly flavour score of 8.55out of 10 was awarded to sample with 10% level, followed sensory score of 8.50 for control sample. The lowest sensory score was awarded to sample containing 20% level of pineapple pulp (8.25 out of 10). The statistical analysis revealed that the effect of addition of different levels of pineapple pulp was significant on flavour of yoghurt samples.

From the results presented in Table 11, it is apparent that the product acidity scores of enriched probiotic yoghurt remains same (1.5 out of 2) for control, sample with 10% and sample with 15% level of pineapple pulp incorporation. The lowest acidity score was awarded to sample containing 20% level, which secured a least sensory score

of 1.0 out of 2. The statistical analysis revealed that the effect of addition of different levels of pineapple pulp was significant on product acidity of yoghurt samples.

5.11. Effect of addition of various fruit pulps on the sensory characteristics of enriched probiotic yoghurt

The highest flavour score (9.25 out of 10), body and texture (4.75) and colour and appearance (4.75) scores were awarded to 15% pineapple pulp sample compared to 15% mango pulp and 15% sapota pulp. The intense mango flavour with slightly thin consistency of 15% mango pulp makes judges to optimize for second preference compared to 15% sapota pulp. Therefore pineapple pulp was preferred by the judges, because, pineapple flavour was closely associated with the yoghurt (sour) taste. The similar findings were made by Ravindra (1985) during development of fruit dahi, Venkateshaiah (1995) during the development of fruit flavoured frozen yoghurt and Dadarwal *et al.*, (2005) during development of fruit flavoured shrikhand.

5.12 Effect of packaging materials on the acidity of enriched probiotic yoghurt stored at 7±1^{0}C

The effect of packaging materials on the acidity of control and enriched probiotic yoghurt stored at refrigeration temperature is presented in Table 13.

The acidity of fresh control yoghurt sample packed in PP, PS and PET remained same (0.85% LA). The acidity of yoghurt packed in PP, PS and PET on second day stored samples were 0.93, 0.94 and 0.87% LA respectively. On 4th day of storage, the acidity of samples packed in PP, PS and PET were 1.09, 1.10 and 0.93 %LA respectively and again there was not much difference in the acidity of samples packed in samples

packed in different packaging materials. On 6th day of storage, the acidity of samples packed in PP, PS and PET were 1.32, 1.36 and 0.98 %LA respectively. The product packed with PP and PS were found to be spoiled on the 6th day, but product with PET container found acceptable. On 8th day of storage, the acidity of samples packed in PET was 1.13 %LA and On 10th day of storage, the acidity of samples packed in PET was 1.54 %LA and not accepted by judges.

The acidity of fresh enriched probiotic yoghurt sample packed in PP, PS and PET remained same (0.82% LA). The acidity of yoghurt packed in PP, PS and PET on second day stored samples were 0.90, 0.91 and 0.80% LA respectively and there was no significant difference between the acidity of samples packed in different packaging material. On 4th day of storage, the acidity of samples packed in PP, PS and PET were 0.95, 0.98 and 0.85 %LA respectively and there was no significant difference between the samples packed in different packaging materials. On 6th day of storage, the acidity of samples packed in PP, PS and PET were 1.07, 1.09 and 0.93 %LA respectively and there was no significant difference between the acidity of samples packed in different packaging materials.

On 8th day of storage, the acidity of samples packed in PP, PS and PET were 1.11, 1.17 and 0.98 %LA respectively and there was no significant difference between the different packaging materials. On 10th day of storage, the acidity of samples packed in PP, PS and PET were 1.19, 1.22 and 1.00 %LA respectively and there was no significant difference between the different packaging materials. On 12th day of storage, the acidity of samples packed in PP, PS and PET were 1.22, 1.26 and 1.03 %LA respectively and

there was a difference in the LA% of samples packed in different packaging materials. On 14th day of storage, the acidity of samples packed in PET was 1.18 %LA. Similarly on 16th day 1.26 %LA followed by 1.58 %LA on 18th day and found spoiled.

5.13. Effect of packaging material on the viability (log_{10} cfu/gm) of probiotic of enriched probiotic yoghurt stored at 7±1^{0}C

The viability of probiotics in the enriched probiotic yoghurt packed on three different packaging materials *viz* PP, PS and PET stored at refrigeration temperature is presented in Table 14.

The results indicate that there was a significant decrease in the viability of *B.bifidum* and *L.acidophilus* counts in the enriched probiotic yoghurt (PP). The *B.bifidum* counts decreased from 7.05 to 6.65 log_{10} cfu/ml and *L.acidophilus* counts decreased from 7.90 to 6.50 log_{10} cfu/ml respectively. Similarly there was a significant decrease in viability of probiotic cultures in enriched probiotic yoghurt (PS). The *B.bifidum* counts decreased from 7.05 to 6.20 log_{10} cfu/ml and *L.acidophilus* counts decreased from 7.90 to 6.10 log_{10} cfu/ml respectively. There was a significant decrease in viability of probiotic cultures in enriched probiotic yoghurt (PET). The *B.bifidum* counts decreased from 7.05 to 6.20 log_{10} cfu/ml and *L.acidophilus* counts decreased from 7.90 to 6.10 log_{10} cfu/gm respectively.

VI. SUMMARY

Attention of many research workers has been drawn for effective utilization of whey protein concentrate (WPC), fruits and probiotic cultures in various food products preparation. This study was undertaken to optimize processing parameters for development of enriched probiotic yoghurt by incorporating probiotic cultures, whey protein concentrate (WPC) and fruit pulps, focusing on its effect on physico-chemical, sensory characteristics, microbiological aspects and shelf-life. This chapter summarizes the outcome of the above said investigation.

6.1 The yoghurt prepared using LS3 combination was found to have higher viable log counts when compared to other two combinations (LS1 and LS2) of cultures.

6.2 Three different combinations of starter cultures tried (at the rate of 3.0 %) for the preparation of yoghurt are

a. *S.thermophilus* + *L.bulgaricus* + *B.bifidum* (1:1:1) (LS1)

b. *S.thermophilus* + *L.bulgaricus* + *L.acidophilus* (1:1:1) (LS2)

c. *S.thermophilus* + *B.bifidum* + *L.acidophilus* (1:1:1) (LS3).

The organoleptic quality of yoghurt prepared using LS3 combination was significantly superior compared to other samples (LS1 and LS2 samples).

6.3 Among the three different heat treatments of milk *viz*, Pasteurization, Sterilization and Boiling, boiled milk yielded a product with superior sensory characteristics when compared to pasteurization and sterilization.

6.4 Among the different heat treatments of milk tried on the quality of the product, product obtained by using boiled milk showed superior quality with respect to flavour, body and texture due to optimal composition. The recovery of total solids was higher in sterilized milk product T3 when compared to pasteurization T1 and boiling T2.

6.5 The effect of different heat treatments of milk did influence on the growth of probiotic cultures like *B.bifidum* and *L.acidophilus*. The viable counts were higher when milk treated with sterilization. But the product was found to be poor colour and appearance and had cooked flavour; hence boiled milk product was optimized.

6.6 Among the four different levels of WPC (0.5, 1.0 and 1.5 % levels) tried for preparation of enriched probiotic yoghurt, WPC at 1.0 % level showed significantly better sensory characteristics with respect to flavour, body and texture, colour and appearance when compared to 0.5, 1.5 % levels.

6.7 The viable counts were higher when WPC was incorporated at 1 % level when compared to 0.5 and 1.5% levels of WPC incorporation.

6.8 Among the three different levels of mango pulp incorporated (10, 15 and 20 % levels) during the preparation of enriched probiotic yoghurt 15 % level of mango pulp showed better sensory characteristics with respect to flavour, body and texture, colour and appearance when compared to 10 and 20% levels.

6.9 Among the three different levels of sapota pulp (10, 15 and 20 % levels) tried for preparation of enriched probiotic yoghurt, 15 % level sapota pulp showed better

sensory characteristics with respect to flavour, body and texture, colour and appearance when compared to 10 and 20% levels.

6.10 Among the three different levels of pineapple pulp (10, 15 and 20 % levels) tried for preparation of enriched probiotic yoghurt, 15 % level pineapple pulp showed better sensory characteristics with respect to flavour, body and texture, colour and appearance when compared to 10 and 20% levels.

6.11 Mango, sapota and pineapple pulps were tried in the preparation of enriched probiotic yoghurt. The pineapple pulp (15% level) was awarded significantly maximum flavour scores when compared to mango pulp and sapota pulp.

6.12 The acidity of both control and enriched probiotic yoghurt samples packed in PP, PS and PET increased significantly at refrigeration temperature. In case of PP and PS control sample was good up to 8 days at 7 ± 1^0C and in case of PET, control sample was good up to 10 days at 7 ± 1^0C. In case of enriched probiotic yoghurt packed in PP, acceptability was 12 days at 7 ± 1^0C, whereas in case of PS enriched sample was found good up to 12 days at refrigeration temperature and in case of PET enriched sample was accepted up to 18 days of storage at refrigeration temperature.

6.13 The viable counts ($\log_{10}$ cfu/gm) of *B.bifidum* and *L.acidophilus* in three different packaging materials like PP, PS and PET containing enriched probiotic yoghurt stored at refrigeration temperature declined significantly during storage period from 0 – 18 days. At 12^{th} day of storage, 10^6 cfu/gm viable cells were retained in the product.

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VIII. ABSTRACT

The consumption of fermented dairy products is increasing in recent years, mainly due to its excellent nutritional and therapeutic properties. Incorporation of probiotic cultures like *B.bifidum* and *L.acidophilus* into the yoghurt would be of paramount importance in making these organisms available to the common population. Whey, which contains nearly 50 % of milk solids is of great significance to utilize WPC in human food chain, because it possess various physico-chemical, nutritional and functional properties. With the growing interest in the diversification of food products to attract wide consumers, in recent past we have noticed that many attempts have been made to incorporate fruit pulps in order to increase the sensory attributes of the product. From this investigation it was concluded that enriched probiotic yoghurt can be prepared by incorporating probiotic cultures (*S.thermophilus*, *B.bifidum* and *L.acidophilus)* in the ratio of 1:1:1 at 3 % level. WPC at 1% level and fruit pulps (pineapple pulp) at 15% level. So thus developed product packaged in PET have improved the sensory characteristics, functional and nutritional properties, with an improved shelf life up to 18 days of storage. In this context the development of enriched probiotic yoghurt has high potential for commercialization thereby it adds to economic development of Indian dairy industry. Further, different combinations of the inoculums of probiotics and incorporation of different probiotics into the milk and their effect on the quality of the product can be studied.