IMMOBILIZATION OF BETA GALACTOSIDASE FOR PRODUCTION OF FERMENTED MILK PRODUCTS WITH LOW LACTOSE

By

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THESIS

submitted in partial fulfilment of the
requirement for the degree

Master of veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Dairy Science

COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy Thrissur

1996

DECLARATION

I hereby declare that this thesis entitled **"IMMOBILIZATION OF BETA GALACTOSIDASE FOR PRODUCTION OF FERMENTED MILK PRODUCTS WITH LOW LACTOSE"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree diploma associateship fellowship or other similar title of any other University or Society

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ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my guide Dr V Prasad Ph D , Associate Professor and Head K A U Dairy Plant Mannuthy It was only because of his keen interest critical advise and constant encouragement during each and every step of my research work that I was able to complete this study successfully

I shall ever be grateful to Dr K Pavithran Ph D Professor and Head, Department of Dairy Science Dr M Mukundan Ph D Associate Professor Department of Dairy Science and Dr G Krishnan Nair Ph D Associate Professor Department of Microbiology for their valuable suggestions and encouragement throughout the study

I am very much thankful to all the staff members and colleagues of Dairy Science Department for their help and co-operation throughout the study

I express my sincere thanks to Dr K C George, Professor and Head and other staff members of Department of Statistics for extending help and guidance in the analysis of data

I gratefully acknowledge Indian Council of Agricultural Research New Delhi for awarding me the Junior Research Fellowship in Dairy Microbiology

I am thankful to Dr A Rajan, Dean, Faculty of Veterinary and Animal Sciences for providing the facilities for study and research

My special thanks to Dr Sreeja Rajmohan, Assistant Professor Department of Dairy Science for the encouragement and providing me the needed help to complete the research work and thesis in time

In this occasion, it gives me great pleasure to thank all the members of KAU Dairy Plant for providing me assistance and co-operation throughout the period of my research work

I take this opportunity to thank my batchmates and friends Dr V Beena, Dr K Raji Dr K P Mini and Km V K Sheena for their encouragement and refreshing company during my stay in the campus

Without the continuous and patient support from my beloved parents and loving sister I would not have been able to complete this work I am greatly indebted to them

Finally, I would like to appreciate and thank Mr O K Ravindran, C/o Peagles, Mannuthy for his elegant typing and suggestions during the preparation of manuscript

GEETHA, R.

To my loving parents

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Introduction

INTRODUCTION

Milk and milk products constitute a major source of animal protein fat carbohydrate many minerals vitamins and other essential growth promoting factors Lactose is a naturally occurring disaccharide of milk that plays an important role in health due to its ability to stimulate intestinal absorption and retention of calcium

The digestion of lactose is dependant on the presence of an enzyme known as lactase or B-galactosidase which is synthesised in the brush border area of the epithelial cells of the small intestine Nearly all newborn full term infants possess sufficient lactase activity to digest lactose However lactase activity declines after weaning and by the age of six only about five to ten per cent of the activity remains It has been estimated that 70 per cent of the world's population experiences reduced activity of intestinal lactase after early childhood (Newcomer and McGill 1984)

Lactose intolerance is a physiological disorder seen in infants and adults Reduction in lactase activity results in accumulation of unhydrolysed lactose in large intestine This increases the osmolarity of intestinal fluids Due to this water is drawn into colon and diarrhoea results A part of the lactose is fermented by the natural flora of intestine

resulting in gas formation and consequently cramping and bloating

The consumption of certain cultured milk products like yogurt bifidus yogurt acidophilus milk etc are suggested as a remedy for gastro intestinal infections lactose intolerant symptoms and also for liver and bile malfunctions

Yogurt is a popular fermented milk product and is believed to be highly nutritious This product is able to provide beneficial effect for lactose maldigesters primarily because the starter culture bacteria contains the enzyme β-galactosidase Bifidobacteria, ~~are~~ one among the natural microflora of human gastro intestinal tract and constitute 99 per cent of the intestinal flora in breast fed infants Considerable beneficial effects have been reported by Colombel *et al* (1987) due to the incorporation of bifidobacteria in regular diet of human beings The most useful media for administering bifidobacteria are yogurt and milk In the case of acidophilus milk the key starter organism *L acidophilus* is able to get implanted in the large intestine of human beings through regular consumption of the product and thereby providing therapeutic benefits

Lactose in milk is relatively insoluble and has a lower sweetening value as compared with sucrose The low solubility

of lactose contributes to its crystallization which is often responsible for a gritty texture in concentrated dairy products Low tolerance and insolubility of lactose have limited its use in foods such as ice cream candy and animal feeds These factors have limited the utilization of whey because of its high lactose content

Although some lactose is metabolised during fermentation yogurt and other cultured milk products still contain appreciable lactose Hydrolysis of part of the lactose in milk prior to the manufacture of various products is a practical solution to the technological problems associated with lactose Enzymatic hydrolysis of lactose using β-galactosidase can result in the formation of soluble and sweeter monosaccharide components glucose and galactose As a result the lactose content in the yogurt mix get reduced and the product become more digestible by lactose sensitive individuals Hydrolysis may provide an added advantage like faster acid development

Industrial β-galactosidase preparations are usually produced from specific strains of bacteria yeasts or moulds Microbial lactases are considered potentially most suitable for the application in food industry The most widely studied enzyme source for food application has been the yeast *K fragilis* Lactose fermenting yeasts have intra cellular

β-galactosidase and are good source of this enzyme There are several patents in all parts of the world on production of lactase from this organism The yeasts have the added advantage of being approved for use in foods

Technologically feasible process for lactose hydrolysis in fluid dairy system include immobilized enzyme reactors membrane reactors with soluble enzyme recycling and direct addition of soluble enzymes Batch hydrolysis with soluble enzyme is the simplest method But high costs of the purified enzyme preparation relative to their single use limits the industrial feasibility of this technique

Immobilized enzymes may be defined as enzymes whose free movement has been restricted in some manner There are several advantages connected with the use of immobilized enzymes for food processing In addition to the possibility of reusing the enzyme the reaction can be easily controlled This gives more precise enzyme reaction and it can be carried out with minimal contamination of food product with added enzyme Above all, the immobilized enzyme can be utilised in continuous operation with great flexibility in reactor design

Enzyme can be immobilized by several techniques The techniques include physical adsorption on organic and inorganic supports entrapment within gel matrix covalent

attachment to an inert support incorporation directly into a polymer and inter molecular cross linking of enzyme molecules Organic supports generally have more binding sites but often do not have the most desirable flow properties and are often excessively affected by factors like pH or deterioration by microorganisms Inorganic supports generally have fewer reaction sites but are more stable Porous supports offer more surface area but present more diffusional problems in reactor In the case of gel entrapment if pores are larger in size the product may diffuse out of the gel

The immobilized whole cells have gained considerable importance during the past few years and have got a number of advantages Whole cell lactase is more than ten fold less expensive than commercially available industrial grade soluble yeast lactase The risk of contamination of final product with yeast can be minimised while using this technique Moreover it is possible to reuse the enzyme system

Use of alginate gels or agar to immobilize cultures for use in food fermentation has recently received attention It is a food grade material used by food industry and, immobilization of cultures using these materials is a rapid and gentle process that minimises cell loss Because of the high biomass that can be achieved with immobilized cultures they are ideal for use in continuous fermentation

Effective utilization of lactose in dairy fluid 'waste streams such as whey and permeates continued to be a serious problem for dairy industry because of low demands and poor financial returns for the product Although newly emerging techniques like reverse osmosis and ultra filtration hold promise to abate the problem of whey disposal their application under Indian condition is cost wise prohibitive

It is calculated that the world production of bulk starter is about two million tonnes Considering the enormous volumes of skim milk that is being used for their preparation the possibilities of utilization of whey as a medium for culture maintenance is of considerable importance The addition of non protein nitrogenous compounds and various skim milk fractions to culture media is often necessary to provide suitable environment for starter organisms Many whey based media for propagating starter cultures have been developed and for this purpose whey permeate obtained by ultrafiltration is being utilized world wide

Whether the interest or concern in removing lactose from the milk products is based on nutritional problem or technological interest the process of lactose hydrolysis must be economically feasible Wide spread application of lactose hydrolysis in small dairy plants depend on the availability of a low technology process and low cost disposable enzymes

Considering the above facts an experiment was designed with the following objectives

(i) Development of an immobilized Beta galactosidase enzyme system using suitable microbial enzyme source and a technique that are readily adaptable to dairy plants involving food compatible reagents

(ii) Utilization of hydrolysed condensed whey as a media for culture maintenance

(iii) Preparation of lactose hydrolysed milk by using immobilized system and its utilization for preparation of selected fermented dairy products

Review of literature

REVIEW OF LITERATURE

2.1 Lactose and milk intolerance

Milk is the universal food for mammalian newborns and is an excellent food for good health It contains all the constituents like fat, protein, lactose trace elements and vitamins (Balasubramanyam *et al* , 1988)

Wong *et al* (1988) reported that bovine milk contains 4 8 per cent lactose which represented approximately 50 per cent of total milk solids

According to Kansal (1990) the lactose content in human milk is considerably higher (seven per cent) and meets 40 per cent of caloric need of the infant

2.1 1 Incidence of lactose intolerance

Rao and Dutta (1978) reported that the enzyme lactase (β-galactosidase) hydrolyses lactose into its two components, glucose and galactose

Garza (1979) reported three types of lactase deficiency

(1) Congenital lactase deficiency - an extremely rare and life threatening condition occurring due to genetic defect

(ii) Primary adult lactase deficiency - an age related decrease in lactase activity

(iii) Secondary lactase deficiency - a temporary state of low lactase activity occurring as a result of injury to intestinal mucosa Lactose intolerance may be defined as a clinical syndrome of abdominal pain, diarrhoea, flatulence and bloating after the ingestion of a standard lactose tolerant test dose i e (two g lactose per kg body weight)

In a study conducted by Garza (1979) it was revealed that prevalence of lactose intolerance was equally great in children and adult population

According to Simoons (1978) the average lactose intolerance amongst Indians were 53 per cent

Tandon *et al* (1981) reported a higher incidence of lactose intolerance among South Indians (66 per cent) when compared to North Indians (27 4 per cent)

Hourigan and Mittal (1983) reported that lactose intolerance prevails about 62 and 80 per cent respectively in Bombay and Calcutta

According to National Dairy council (1985) 70 per cent of the World's population experiences reduced intestinal lactase activity after early childhood

Savaiano and Levitt (1987) hypothesised that lactose intolerance was a genetically linked triat

2 1 2 Cultured milk products in relation to lactose intolerance

According to Kon (1972) fermented milks have been consumed by the people throughout the world particularly in areas where lactase deficiency and lactose intolerance were prevalent

Hersh (1972) recommended that reduction of lactose in the diet of intolerant individuals were possible by modification of milk by enzymatic hydrolysis

In several studies conducted on lactose intolerant individuals, it was observed that lactose ingested in yogurt was more effectively digested than lactose in milk (Kilara and Shahani 1976 Savaiano *et al* 1984 Gilliland 1985 and McDonough *et al* 1987)

According to Tamime (1977) the carbohydrate in yogurt is more digestible than that in milk since upto half of lactose is hydrolysed during manufacture

Nutritional and therapeutic qualities of fermented dairy products were studied by Khem *et al* (1979) and reported that B-galactosidase present in fermented products helped the lactose intolerant individuals Digestibility and hypocholesteraemic effect was found to get improved due to the consumption of fermented products

In a study conducted on cultured products by Savaiano and Levitt (1987) it was observed that feeding pasteurized yogurt containing trace amounts of microbial B-galactosidase activity resulted in three fold increase in lactose malabsorption as compared with feeding yogurt containing viable culture They also reported that improved lactose digestion can occur due to autodigestion by microbial B-galactosidase which get released from yogurt cultures by gastric or bile acid digestion

Su *et al* (1992) conducted a survey of the composition of commercial cultured milk products and the results showed that cultured milk products contained 77 13 $\pm$ 0 56 per cent moisture 2 94 per cent milk fat, 4 01 per cent protein, 1 08 per cent acidity, 0 13 per cent lactose and a lactic count of more than 10^7 cfu/ml

Sarkar (1993) advocated that cultured milk products can be used in weaning foods and recommended them for feeding healthy and lactose intolerant infants

2.2 Utilization of whey

2 2 1 Composition of cheese whey

Nutting (1970) reported that cheese whey contained seven per cent total solids which was about half the total solids of milk This contained 4 9 per cent lactose, 0 9 per cent protein 0 3 per cent fat and 0 6 per cent ash

Palmer (1980) reported that lysine content of whey protein comes around 10 9 per cent as against 7 6 per cent in casein Percentage of cysteine in whey protein and casein were 3 15 and 0 4 respectively The total essential amino acids in whey was higher (62 55 per cent) than in casein (49 45 per cent)

The chemical composition of cheese whey and paneer whey was studied by Balasubramanyam *et al* (1988) and reported that the cheese whey solids contained more protein and lactose while paneer whey showed higher fat and ash content

2 2.2 Whey based culture media

Many whey based media for propagating cheese starter cultures have been developed Yeast extract was a commonly used supplement in these media to provide additional growth factors (Ausavanodam *et al* 1977 Richardson *et al* 1977)

Chen and Richardson (1977) reported that the activity of cultures in phosphated acid whey medium (PWM) with pH control was higher than in commercial phage inhibitory medium

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

Wright and Richardson (1982) reported that when the whey based media was fortified with 0 71 per cent yeast autolysate and 0 43 per cent casein hydrolysate there was an increase in cell mass by 36 per cent and culture activity by 38 per cent as compared to control medium fortified with 0 4 per cent yeast autolysate and 0 1 per cent casein hydrolysate

Pahwa and Mathur (1983) observed that inclusion of higher amounts of whey in commercial infant formula increased Bifidus activity by 50 per cent

Christopherson and Zottola (1989) studied the growth characteristics of three lactic streptococci in five different types of whey based media and suggested that all the media could support the growth of mesophilic lactic acid bacteria They also observed that the acid development was more

dependent upon the type of streptococcal strain the temperature of incubation and the day of propagation

Sandine *et al* (1990) developed a whey based bacteriophage inhibitory bulk starter medium for growing thermophilic lactic acid bacteria

Chiarini and Mara (1990) suggested whey as a good natural medium for the growth of lactic acid bacteria responsible for the production of lactate

Gorre *et al* (1992) in their attempt to produce a low cost medium for production of concentrated *B bifidum* found a fifteen fold improvement in batch productivity when whey was used as a culture medium

Modler and Villa Garcia (1993) developed an inexpensive whey based medium for large scale production of bifidobacteria This medium contained 11 per cent whey solids 0 05 per cent cysteine and 0 23 per cent yeast extract They got a count of 10^{9} cfu/ml

Gupta and Gandhi (1995) reported that supplementation of whey with yeast extract and lactose stimulated the growth of lactic acid bacteria and increased the acid production However *L bulgaricus* produced different levels of acidity in whey based media depending upon strains (Reddy *et al* 1976)

The performance of *L delbrueckii* and *L plantarum* were found to be poor in whey based medium irrespective of longer incubation period and high temperature as required by these cultures (Aksu and Kutsal 1986 Chiarini and Mara 1990)

Magdalenic and Krdev (1990) suggested that addition of whey retentate to milk retentate could result in better growth of *B bifidum* and reduced fermentation time

Laund *et al* (1992) observed that yield of lactic acid varied from one strain of lactic acid bacteria to another when grown in media containing whey as a substrate

2.3 Importance of lactose hydrolysis

According to Thompson and Guyriesek (1974) the product prepared from lactose hydrolysed milk was more sweet and acceptable in flavour than those obtained from lactose unhydrolysed milk

Mahoney *et al* (1975) reported that the low solubility of lactose resulted in crystallization and gritty texture in concentrated dairy products

Paige *et al* (1975) recommended hydrolysis of lactose for improving nutritional quality of milk

Bouvy (1975) reported that lactose hydrolysis could improve product desirability especially solubility and sweetness

Lactose hydrolysed concentrated milk was found to possess more protein stability than unhydrolysed milk (Holsinger and Roberts 1976)

Mahoney and Whitaker (1977) suggested the hydrolysis of lactose content in whey and ultrafiltered permeate as a means of utilization of cheese industry waste products

According to Singh and Patel (1985) the solubility of lactose at room temperature was comparatively low and resulted in grainy texture in frozen dairy products They suggested that by hydrolysing lactose more solids could be incorporated in frozen products without the problem of lactose crystallization

Whalen *et al* (1988) hypothesized that hydrolysed milk possess many advantages such as increased sweetness more flavour acceptance faster acid development or shorter coagulation time and increased digestibility

Thompkinson *et al* (1991) reported that low tolerance and insolubility of lactose limited its use in foods such as ice

cream candy and animal feeds and limited the utilization of whey also

Gielman (1993) reported the properties of syrups made by hydrolysis of lactose According to him lactose had a low sweetening potential limited solubility and formed large crystals

2.4 Sources of β-galactosidase

Since microorganisms are the most ideal source of the enzyme B-galactosidase, several workers have screened a large group of organisms for their enzyme activity (Feniksova *et al* 1968 Blankenship and Wells 1974 Sorensen and Crisan 1974)

Wierzbichi and Kosikowski (1973) used β-galactosidase from *Aspergillus niger* for hydrolysis of acid whey and reported that fungal enzymes with acid pH and high optimum temperature were suitable for lactose hydrolysis

Propova *et al* (1974) used β-galactosidase of *S fragilis* for hydrolysis of lactose from milk

Mahoney *et al* (1975) studied the biosynthesis of lactase during the growth of *K fragilis* and reported that maximum enzyme yield was attained at the beginning of stationary phase

of growth Best lactase yield was found to be obtained in a medium containing 15 per cent lactose and at an aeration rate of 2 millimoles of oxygen per litre per minute

Lactase is available in powdered form in small envelopes to the consumers in country like USA and the consumers get rid of the problem of lactose intolerance by adding this enzyme in a glass of milk a day before consumption (McCormick 1976)

Sonawat *et al* (1981) studied the characteristics of β-galactosidase from *K fragilis* β-galactosidase content in cell free enzyme extract was found to be maximum just before the stationary phase of growth at a pH of 4 0 and in the presence of eight to twelve per cent whey solids

Hussein *et al* (1989) reported that B-galactosidase from *S fragilis* produced peak activity at pH 6 8 to 7 2 and at 30 C and that 40 per cent of the lactose in condensed whey got hydrolysed within four hours by this enzyme

Balasubramanyam (1988) reported that a number of food grade enzymes are available from various microorganisms like *K lactis* (Maxilact) *K fragilis* (Lactozyme, Hydrolact) *A niger* (lactose N) and *A oryzae* (Lactosin AO) He also suggested that the hydrolysis of lactose could be carried out by the addition of food grade β-galactosidase at selected time temperature combination

2 4 1 β-galactosidase specific activity

Mahoney *et al* (1975) observed that 41 strains of *K fragilis* varied 60 fold in their ability to produce B-galactosidase According to them the best extraction of lactase from fresh yeast cell was possible by toluene autolysis at 37 C in 0 1 M potassium phosphate buffer and at a pH of 7 0 and they reported a mean specific activity of 3 11 units for *K fragilis*

But according to Rao and Dutta (1979) the specific activity of crude enzyme preparation from *S thermophilus* was three times more than the enzyme preparation from *K fragilis*

Yogurt possessed considerable lactase activity due to the presence of lactase in yogurt organisms *S thermophilus* contained approximately three times more lactase activity than did *L bulgaricus* The lactase activity was 1 5, 2 4 and 3 8 units/gram for *L bulgaricus, S thermophilus* and combined cultures respectively (Savaiano and Levitt 1987)

Lin *et al* (1989) demonstrated a method for determining β-galactosidase specific activity of yogurt cultures in skim milk Under optimal assay conditions the specific activity was found to be 4 5

Modler and Villagarcia (1993) recommended that β galactosidase from *K lactis* required manganese and potassium ions for its complete activation They also observed that the enzyme showed stability over a pH range from 6 0 to 8 0 and was rapidly inactivated below pH 8 0 and at a temperature in excess of 55 C

Stred'ansky *et al* (1993) reported that in optimal conditions (37 C pH 9 5 to 10 5) greater than 90 per cent of the intracellular β-galactosidase activity was released into 0 1 to 0 5 M phosphate buffer after 1 5 to 2 0 hour treatment with one per cent chloroform

2 4 2 Whole cell yeast lactase

Bergess and Kelly (1979) reported that lactose hydrolysis in dairy products without reuse of lactase was not economical and for this problem they suggested the use of whole cell lactase They hypothesised that the whole cell yeast lactase was less expensive than commercially available industrial grade soluble yeast lactase

Brodsky and Grootwassink (1986) reported that the effectiveness of whole cell yeast lactase in hydrolysing lactose content in cheese whey could be assessed by estimating glucose content

Stred'ansky *et al* (1993) suggested that lactose fermenting yeasts have intracellular β-galactosidase and are good source of that enzyme They also reported that the enzyme isolated from yeast has the advantage of being approved for use in foods

2 4 3 Permeabilization of yeast cell wall

Mahoney and Whitaker (1977) suggested manganese chloride 0 15 mM as a precautionary measure in the permeabilization process because of its role in lactase stabilization

But according to Brodsky and Grootwassink (1986) no manganese chloride was required for either lactase stability or for cell permeabilization They also observed that yeast cell membranes were more sensitive to permeabilization with increasing pH But at a pH of 7 3 or below the enzyme showed stability

Brodsky and Grootwassink (1986) recommended the "puncturing of cell membrane" for activating the hydrolytic capacity of intra cellular lactase

2.5 Immobilization of β-galactosidase

The first reported case of immobilized enzyme was almost 70 years ago (Nelson and Griffin 1916) A major impetus to

the development of immobilized enzyme technology occurred in the mid to late 1960s

According to Woychik and Wondolowski (1973) one time use of β-galactosidase in free form was uneconomical and they proposed immobilization technique for continuous extended use of the enzyme

Advantages of immobilized enzyme as reported by Kilara (1979) included repeated use of a single batch of enzyme, better process control, enhanced stability, adaptability to continuous use and good quality product free from enzyme

Hultin (1983) suggested the following advantages connected with the use of immobilized enzymes

(i) Possibility of reusing the enzyme
(ii) More precise control of the reaction
(iii) Continuous operation of the system and
(iv) More safer use

Thompkinson (1989) reported that immobilized enzyme system could be used repeatedly and the process could be made continuous with a small amount of enzyme

Sridhar and Dutta (1991) observed that β-galactosidase when used in soluble form could not be reused and they

suggested that by adopting immobilization technique the process could be run continuous with least contamination of the product

2 5 1 Various immobilization techniques

Olson and Stanly (1973) immobilized Beta galactosidase by adsorption on phenol formaldehyde resin and found it to be an excellent support for immobilization of enzyme

Immobilization of Beta galactosidase of *Aspergillus niger* by adsorption on stainless steel and nickel oxide activated with a layer of titanium oxide was reported by Hasselberger *et al* (1974)

Immobilization of Beta galactosidase on various other supports like collagen (Giacin *et al* 1974) feather protein (Stanley *et al* 1975), granular carbon (Lin *et al* , 1975) hen egg white (Kaul *et al* 1984) have also been reported

Proceedings of the first National Seminar on immobilized enzyme engineering held in 1979 at Jadavpur University discussed about the hydrolysis of lactose in whey and milk by immobilized whole microbial cells and its application in food industry

Linko *et al* (1981) immobilized living *K fragilis* cells on calcium alginate gel beads at a level of four to 16 gram yeast per gram of sodium alginate In batch system about 90 per cent conversion was obtained in 48 hours both with free and immobilized yeast lactase using whey containing five to ten per cent lactose

Hultin (1983) suggested that enzymes can be immobilized by several techniques which include physical adsorption entrapment within a gel matrix covalent attachment to an inert support incorporating directly into a polymer and by intermolecular crosslinking of enzyme molecule

Sridridenko *et al* (1985) conducted study about hydrolysis of lactose in whey using immobilized B-galactosidase Four ml of immobilized B galactosidase with 250 to 270 units activity per ml were placed in a column and the reaction was allowed to carry at optimum pH and temperature In this reaction more than 80 per cent of the lactose got hydrolysed

Morris (1986) used immobilized enzymes for treatment of milk cheese whey and whey permeate

Steelson *et al* (1987) reported that immobilization of cultures in alginate gel was a rapid and gentle process that minimized the cell loss Because of the high biomass that

could be achieved with immobilized cultures they suggested that the above technique was ideal for use in continuous fermentation

Rao *et al* (1988) immobilized the yeast cells (*K fragilis*) using standard techniques Yeast cells were mixed with two per cent aqueous solutions of sodium alginate and the mixture was extruded through a syringe into 0 1 M calcium chloride to produce beads of three mm diameter to be used for lactose hydrolysis

Decleire *et al* (1985) conducted immobilization studies on *K bulgaricus* using calcium alginate beads and hen's egg white containing two per cent gluteraldehyde Maximum hydrolysis was observed when alginate column was used (88 per cent) whereas in the latter only about 74 per cent hydrolysis was observed They also reported that the cells immobilized on egg white retained 60 per cent enzyme activity after 24 days of continuous operation at 4 C

Gekas and Leiva (1985) advocated immobilized enzyme reactors as technologically feasible process for lactose hydrolysis

In a study conducted by Rao *et al* (1988) ten g immobilized cells of *S fragilis* completely hydrolysed 100 ml

of milk within 3 5 hours The system was used repeatedly in 15 batches without change in activity

Rao *et al* (1988) reported the following advantages for whole cell immobilization

(i) Retention of high cell densities and faster reaction rate

(ii) Minimisation of contamination of the product with yeast
(iii) Rapid hydrolysis of lactose in milk and
(iv) Repeated use of the enzyme system

Sharma and Dutta (1990) reported that immobilization of enzyme helped to keep the enzyme separate from the product and enabled its reuse

2.6 Fermented milk products

2 6 1 Yogurt and bifidus yogurt

Technology of bifidus culture in the milk processing industry and the application of bifidus culture in the manufacture of fermented milk was first described by Shuler-Mayloth *et al* (1968)

For the satisfactory growth of *B bifidum* Anand *et al* (1984) suggested the addition of one per cent dextrose and

0 1 per cent yeast extract in milk whereas Collins and Hall (1984) advocated the supplementation of cysteine and pyruvic acid

According to Tamime and Robinson (1985) both short set (40-45 C for 2 1/2 to 3 1/2 Hours) or long set (30 C for 18 hours) method of incubation of yogurt could be practised under industrial condition depending upon type and method of subsequent cooling

Scardovi (1986) reported that the optimum growth temperature for majority of bifido bacterium species was 37 to 41 C However they could grow at 25 to 28 C and was able to tolerate a temperature upto 45 C Optimum pH for initial growth was found to be 6 5 to 7 0

Desjardins and Roy (1990) recommended yogurt as the most useful media for administering bifido bacteria

Beena (1995) recommended bifidus yogurt particularly fortified with lactose hydrolysed condensed whey for patients with high cardiac risk factor arising from high serum total cholesterol She suggested that lactose hydrolysed condensed whey and *B bifidum* could be successfully incorporated in yogurt for improving therapeutic benefits in terms of increased β-galactosidase specific activity and hypocholesteraemic effect

2 6 1 1 Influence of lactose hydrolysis on physico chemical properties of products

Wendorff *et al* (1971) reported that as the percentage of lactose hydrolysis in the product increased, the moisture content and bulk density decreased

Woodward and Kozikowski (1975) observed that the body and texture and flavour scores were better for cheese prepared with lactose hydrolysed milk than those made with normal procedure

Thompson and Brower (1976) also supported that prehydrolysis of lactose could improve the body, texture and flavour of cheese

According to Gyuriesek and Thompson (1976) as the percentage of lactose hydrolysis increased the time of curd setting decreased by about 40 minutes

O'Leary and Woychik (1976) reported that the lactose hydrolysed yogurt had more lactic acid than in control Flavour score was also found to be significantly higher in lactose hydrolysed milk

Labuschange and Nienwondt (1978) reported that addition of β-galactosidase reduced the manufacturing time, in addition to enhancing the rate of ripening of cheese

Marschke and Dulley (1978) reported a faster acid development in lactose hydrolysed milk than control

Mahoney and Adamchuk (1980) observed that when the percentage of lactose hydrolysed whey utilized in ice cream manufacture was increased, there was an increase in freezing time and firmness of the product However viscosity, freezing temperature and pH were found to get decreased

According to Nijpels (1981) lactose hydrolysed milk could provide a firmer curd and increas the yield of dairy products

Prakasha and Sharma (1984) observed sweetness and browning in Khoa made from lactose hydrolysed buffaloe milk

Abdou-Sonia *et al* (1984) observed that use of lactose hydrolysed milk in the preparation of Kareish cheese decreased coagulation time by 6 7 to 10 per cent and increased cheese yield by 15 4 to 17 9 per cent Sensory scores were also found to be higher in products prepared with lactose hydrolysed milk

Lindamood and Grooms (1989) reported that hydrolysis of lactose and sucrose increased sweetness but decreased freezing point and firmness of ice cream But hydrolysis did not affect organoleptic properties and melt down characteristics of the product

Hydrolysis offered many technological advantages such as improved solubility and increased sweetness of glucose and galactose compared with lactose Harju (1993) He also reported that the improved absorption of hydrolysed lactose in human small intestine and prevention of lactose intolerance were the commercially most important benefits

According to Baig and Prasad (1995) incorporation of whey solids in yogurt was stimulatory to the growth of *S thermophilus* and *B bifidum*

2.6 1.2 Proteolytic activity

Biochemical properties of certain selected strains of lactic acid bacteria were studied by Dutta *et al* (1971) and reported a proteolytic activity of 0 26 mg/g for *S thermophilus*, 0 28 mg/g for *L delbrueckii* sub sp *bulgaricus* and 0 18 mg/g for *L lactis*

Tamıme and Deeth (1980) reported that proteolysıs ın yogurt was dependent upon the strain of yogurt culture and the ratıo between thermophılus and bulgarıcus

According to Marschke *et al* (1980) addıtıon of 'maxılact' to cheese mılk resulted ın ıncreased proteolytıc breakdown ın cheese

Grooda *et al* (1983) also observed an ıncrease ın proteolysıs ın the enzyme treated cheddar cheese

Shankar *et al* (1983) reported that the tyrosıne value of yogurt prepared wıth fresh mılk was 23 1 mg/100 g

Tamıme and Robınson (1985) reported the pattern of proteolysıs by yogurt cultures *L delbrueckıı* sub sp *bulgarıcus* possessed more proteınase actıvity and hydrolysed casein to form poly peptıdes The peptıdases synthesısed by *S thermophılus* was responsıble for breakıng of the peptıdes and lıberatıon of free amıno acıds

According to Slocum *et al* (1988) the amount of free amıno acıds ın the mılk after the fermentatıon by a sıngle culture of bulgarıcus were hıgher than ın the product ın whıch fermentatıon was carrıed out by mıxed cultures of thermophılus and bulgarıcus

According to Kurmann (1988) bifido bacteria had a very weak proteolytic activity Among this species considerable strain variation was also observed

Rajagopal and Sandine (1990) reported a high proteolytic activity for *L bulgaricus* (61 to 144 ug/ml) when compared to *S thermophilus* (2 4 to 14 8 ug/ml) But mixed cultures showed the highest tyrosine value of 92 6 to 419 9 ug/ml

Abraham *et al* (1993) studied the proteolytic activity of *L bulgaricus* grown in skim milk at different temperatures They reported the highest proteolytic activity when grown at a temperature of 34 to 38°C The activity was found to be decreased at growth temperature above 40 C

Geetha and Khan (1994) studied the influence of lactic acid bacteria on proteolytic activity and reported that *L bulgaricus* was highly proteolytic (258 Ng/ml) followed by *L acidophilus* (224 ug/ml) and *S thermophilus* (95 33 Ng/ml)

2 6 1 3 Viable count

Misra and Kuila (1991) studied the efficiency of various media for enumeration of *B bifidum* in fermented milks Use of phosphate buffer as diluent and yoshioka agar with 0 1 ml sodium thioglycollate (15 per cent w/v) in every petriplate

and on incubation for 72 hours at 37 C resulted in maximum growth of the organism

Ishibashi and Shimamura (1993) reported that cultivation of *S thermophilus* with *B bifidum* would be beneficial because carbondioxide released by *S thermophilus* can be stimulatory for bifidobacterium

Baig (1994) suggested that the growth of *S thermophilus* could be stimulated by incorporation of *B bifidum* as a supplementary culture He recorded an optimum growth of *B bifidum*, when growth in association with *S thermophilus* and *L bulgaricus* and the range of *S thermophilus* *L bulgaricus* and *B bifidum* were 3 06x10⁹ to 4 26x10⁹ 2 36x10⁹ to 3 91x10⁹ and 2 25 to 3 48x10⁹ cfu/ml respectively

2 6 1 4 Effect of lactose hydrolysis on culture activity

Dutta *et al* (1971) reported an acidity percentage of 0 67 for *S thermophilus* 1 41 for *L delbrueckii* sub sp *bulgaricus* and 0 61 for *L lactis* The corresponding pH values reported were 5 1 4 0 and 4 5 after 24 hours of incubation at their optimum temperature in ordinary skim milk

According to Abd-El-Hady *et al* (1985) lactose hydrolysis had an inhibitory effect on the growth of mixed cultures

Abd-El-Hady *et al* (1985) conducted a study on the effect of adding lactase enzyme on the growth and activity of some dairy microorganisms and reported a slight increase in acid development and in the count of *L lactis* in lactose hydrolysed milk An inhibitory effect on the growth of mixed cultures of *S thermophilus* and *L bulgaricus* were also observed as a result of lactose hydrolysis

Arsov (1988) conducted fermentation studies on enzymatically hydrolysed milk inoculated with two sets of yogurt cultures separately It was found that the activity of *S thermophilus* was greater in hydrolysed milk than in unhydrolysed milk, in the case of first culture tested Acid production and activity of *S thermophilus* and *L bulgaricus* were found to be similar with the second culture used

Oh *et al* (1991) observed a slight increase in viable cell numbers and in acid production of *B bifidum* and *L acidophilus* when inoculated separately in lactose hydrolysed milk whereas in the case of fermentation with mixed cultures there was no effect on viable cell count and acid production in control and B-galactosidase treated milk

2 6 2 Acidophilus milk

During the past 80 years attention had been directed on the benefits derived from consumption of milk products

containing *Lactobacillus acidophilus* Introduction of acidophilus milk in Indian diet had vital scope since the product had more therapeutic value as compared to dahi (Gandhi and Rao 1989) They defined acidophilus milk as a sour milk product where milk had been allowed to ferment under conditions that favour the growth of *L acidophilus*

2 6 2 1 Therapeutic properties of Acidophilus milk

Silver (1961) reported that *L acidophilus* based fermented milk products could be used to cure skin infection aphtheous stomatitis and some other ailments The organism had been able to control gastro intestinal disorders such as diarrhoea, constipation, dyspepsia, flatulence, colitis and several other alimentary disorders in adults as well as children

L acidophilus is known to constitute a dominant part of the normal intestinal microflora living in association with a number of other groups of microorganisms like coliforms, streptococci and bacterioids It was reported that it can exert a regulatory influence on the relative proportions of different species (Donaldson, 1968)

Nielson (1979) recommended *L acidophilus* as the only species of the lactobacilli capable of colonising in the intestinal tract and of producing the beneficial effects He

reported that some bacteriocins produced by this organism retarded the growth of many of the type of bacteria causing intestinal disorders in man

Gilliland (1989) suggested that *L acidophilus* had a potential for preventing the intestinal infection, improving lactose digestion in lactose intolerant people, able to control serum cholesterol level and having anticarcinogenic activity

2 6.2 2 Compositional characteristics for acidophilus milk

Rao and Gandhi (1987) observed a titratable acidity of 1 0 to 1 2 per cent of lacticacid for acidophilus milk prepared with pure cultures of *L acidophilus* at 2 5 per cent level, incubating at 37°C for eight hours

Rao and Gandhi (1987) also suggested the following compositional characteristics for acidophilus milk Titratable acidity percentage of 1 05 to 1 19, Total solid percentage of 16 53 to 16 92, pH of 3 9 to 4 1 and viable count of $64x10^7$ to $81x10^7$ cfu/ml

Yadav **et** *al* (1993) reported a titratable acidity of one per cent and viable count of 2000 to 3000 million/ml for acidophilus milk as standard requirements

Legends are given between pages 165 and 166

Materials and Methods

MATERIALS AND METHODS

3.1 Starter cultures

The following pure freeze dried cultures were procured from National Dairy Research Institute (NDRI), Karnal

(i) *Streptococcus thermophilus* YH-S

(ii) *Lactobacillus delbrueckii* sub sp *bulgaricus* YH-L

(iii) *Lactococcus lactis*

(iv) *Lactobacillus acidophilus* - AH 1

Yeast culture, *K fragilis* was obtained from NDRI, Karnal in the form of slant culture

Bifidobacterium bifidum 2715 in the form of freeze dried state was obtained from National Collection of Food Bacteria, England (Agricultural and Food Research Council (AFRC), Institute of Food Research, Reading Laboratory, Reading RG6, 2EF, UK)

3.1 1 Maintenance of starter cultures

The cultures namely *S thermophilus L delbrueckii* sub sp *bulgaricus L acidophilus L lactis* were activated by three consecutive transfers in sterile skim milk for maximum

activation These cultures were then maintained by transfering in sterile skim milk at fortnightly intervals

Initial activation of *B bifidum* was achieved by transferring the contents of the ampoule aseptically into sterilized skim milk containing one per cent dextrose and 0 1 per cent yeast extract and incubating at 37 C for 48 hours under carbon dioxide tension After initial activation three more transfers were done in the same media to make the culture active and to get curdling within 24 hours using a two per cent inoculum Further maintenance of this was done by subculturing in sterilized skim milk every fortnight using two per cent inoculum and incubating at 37 C for 24 hours

K fragilis obtained in the form of slant culture was maintained by propagating in agar medium containing one per cent lactose and having the following composition

Lactose	-	10 g
Peptone	-	5 g
Beef extract	-	3 g
Sodium chloride	-	5 g
Agar	-	15 g
Distilled water	-	1000 ml

pH adjusted to 7 0

Between transfers all the above cultures were stored at 4 C

3.2 Preparation of immobilized enzyme system

3 2 1 Collection of cell mass

Lactose broth was used to propagate the cultures of *S thermophilus*, *L delbrueckii* sub sp *bulgaricus* and *K fragilis* In 500 ml conical flasks lactose broth was taken at 250 ml level and sterilized by autoclaving at 115°C at 10 lbs pressure for 30 minutes Cooled the samples to 37 C and inoculated with active cultures of *S thermophilus* and *L delbrueckii* sub sp *bulgaricus* separately Incubated the samples at 37 C for 24 hours whereas active cultures of *K fragilis* was inoculated into lactose broth, after cooling to 30 C and incubated at 30 C for 24 hours Sufficient air space was provided in the flask in order to favour the growth of the yeast During incubation 25 ml of 10 per cent sterile lactose solution was added at six to eight hour intervals in order to maintain the lactose concentration at one per cent level

After 24 hours of incubation of each cultures at their optimum temperature centrifuged the media at 8000 rpm for 15 minutes using a refrigerated centrifuge and harvested the cell growth These cell pellets were transferred to separate

sterile freeze drying flasks and freeze dried The dried materials were then transferred to a sterile screw capped vials and stored in deep freeze until further use

3 2.2 Permeabilization of cells

Reagents

(i) 0 1 Molar (M) dihydrogen phosphate solution

Prepared by dissolving 1 39 g sodium dihydrogen phosphate in one litre distilled water (Reagent 1)

(ii) 0 1 M disodium monohydrogen phosphate solution

Prepared by dissolving 2 682 g disodium hydrogen phosphate in one litre distilled water (Reagent II)

(iii) 0 1 M phosphate buffer

Prepared by mixing 81 5 ml of reagent I and 18 5 ml of reagent II and by making up the volume to 200 ml with distilled water

Procedure

One gram freeze dried cells of *S thermophilus L delbrueckii* sub sp *bulgaricus* and *K fragilis* were taken separately in three sterile conical flasks and 100 ml of

0 1 M phosphate buffer (pH adjusted to 6 2) was added Five millilitres of 0 3 per cent potassium sorbate was added to the above suspensions and agitated at 60 rpm at 20 C for 30 minutes in order to achieve permeabilizaton

After permeabilization of the cells the permeabilizing agent potassium sorbate was removed by centrifugation at 8000 rpm for 15 minutes, discarded the supernatant and collected the cell mass

3 2 3 β-galactosidase specific activity of cultures

Permeabilized cells of *S thermophilus*, *L delbrueckii* sub sp *bulgaricus* and *K fragilis* were screened for their β-galactosidase specific activity as per the procedure described by Lin *et al* (1989) The detailed procedure is given below

3 2.3 1 Preparation of cell free enzyme extract

One gram of permeabilized cells were suspended in one millilitre of 20 mM phosphate buffer (20 mM containing 5 mM magnesium sulphate - pH 7 0) and washed twice These cell pellets were resuspended in 10 ml of phosphate buffer and sonicated for three minutes one minute each, with sufficient interval, using Imeco ultrasonic sonifier

Samples were maintained on ice throughout the procedure to prevent enzyme denaturation by the heat generated during sonication Then the cell suspension was centrifuged at 11,400 x g for 10 minutes to remove the cell debris and whole cells The supernatent cell free enzyme extract was immediately assayed for B-galactosidase specific activity and protein content

3 2 3 2 β-galactosidase assay

β-galactosidase activity was measured using a chromogenic substrate 0-nitrophenyl-B-D galactopyranocide (ONPG) Reaction mixtures were prepared by mixing four millilitres of thirty two micromoles of ONPG solution and one ml of cell free enzyme extract as above The reaction mixture was incubated at 37 C in a waterbath for 30 minutes Colour development at the end of incubation was measured at 420 nm, using a spectronic-20 spectrophotometer Total ONP released was calculated by interpretation with a standard curve (standard curve was prepared by dissolving 0-Nitrophenol in minimum quantity of alcohol and making up the volume by phosphate buffer to give concentrations two to 48 micromoles/ml The optical density of each concentration was measured in a spectronic-20 spectrophotometer at 420 nm

3 2 3 3 Protein assay

The protein content of the cell free enzyme extract was estimated using the procedure described by Lowry *et al* (1951)

Reagents

(i) Four per cent sodium carbonate in distilled water (Reagent I)

(ii) 0 5 per cent copper sulphate in one per cent potassium sodium tartrate in distilled water (Reagent II)

(iii) Alkaline copper reagent was prepared by mixing 50 ml of Reagent I with two ml of Reagent II

(iv) 0 1 N sodium hydroxide

(v) Diluted Folin reagent (Folin reagent was diluted with equal volume of 0 1 N sodium hydroxide)

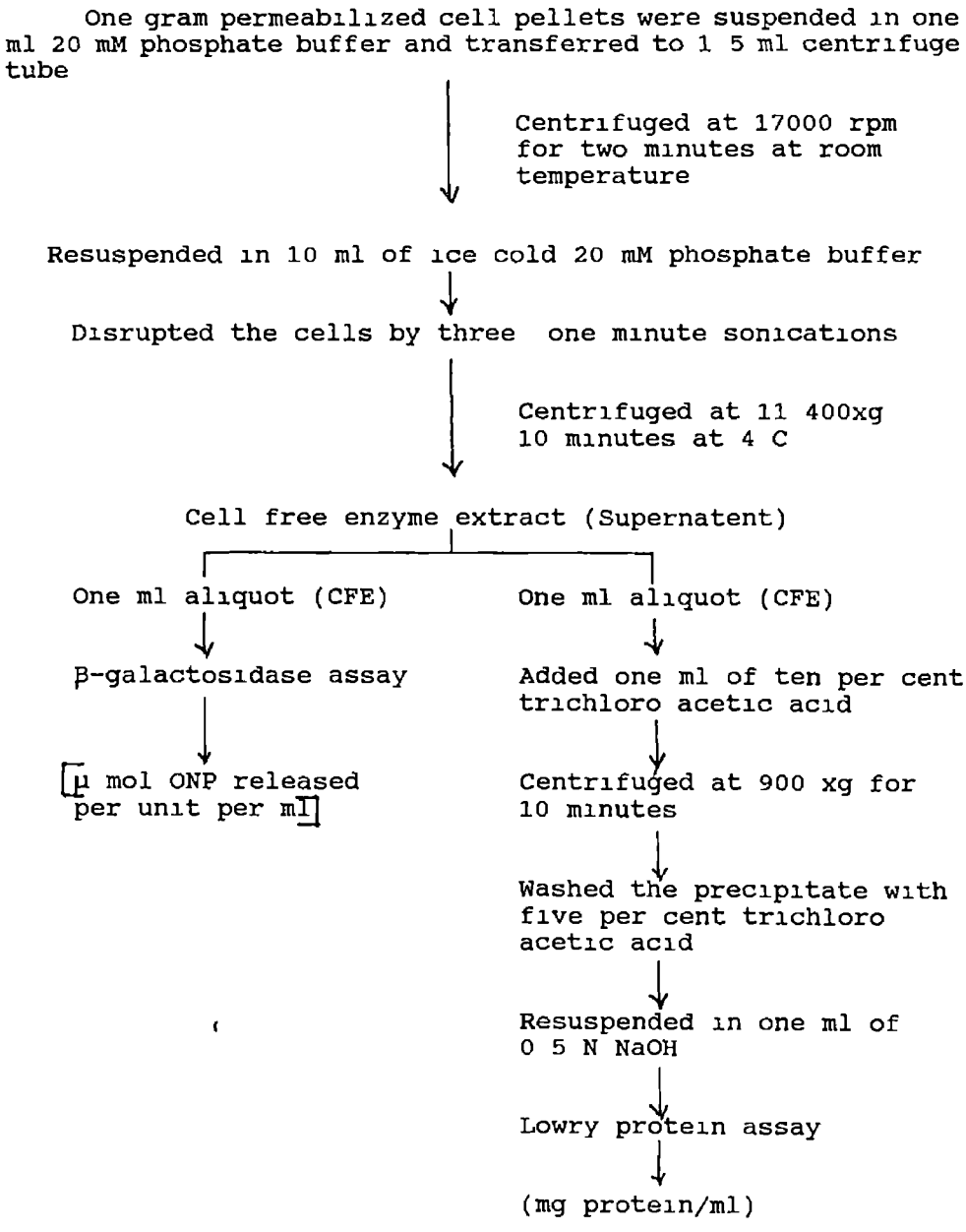
(vi) Standard protein solution (Bovine serum albumen 100 g/ml)

One millilitre of cell free enzyme extract (CFEE) was added with one ml of 10 per cent Trichloro acetic acid (TCA) and centrifuged at 900xg for 10 minutes at 4 C Supernatent

was discarded and the protein precipitate was washed twice with five per cent TCA and resuspended in 0 5 N sodium hydroxide solution (one ml) To this 1 5 ml of alkaline copper reagent was added It was shaken well and allowed to stand for 10 minutes, after which exactly 0 15 ml of diluted Folin reagent was added with continuous shaking The tubes were allowed to stand for 30 minutes Then the colour development was read at a wavelength of 500 nm The protein was measured by interpreting the value with a standard curve

(Standard curve was prepared by dissolving bovine serum albumen in distilled water to get concentrations from 25 µg/ml to 400 µg/ml Each concentration was used for colour development and the optical density was measured at 500 nm The values were plotted in a graph)

Flow diagram for β-galactosidase specific activity



$$\beta\text{-galactosidase specific activity} = \frac{\mu \text{ mol ONP released}}{\text{mg protein}}$$

Based on the β-galactosidase specific activity, one organism was selected for further studies

3 2 4 Immobilization of the cells

Reagents

(i) 0 1 M phosphate buffer

(ii) Two per cent food grade agar

Prepared by dissolving two g of food grade agar in 100 ml distilled water

(iii) Two per cent sodium alginate

Prepared by dissolving two grams of sodium alginate in 100 ml distilled water

(iv) Toluene

All the above reagents were sterilized by autoclaving at 121°C at 15 lbs pressure for 15 minutes before used for immobilization technique

Procedure

One gram of permeabilized cells of *K fragilis* was taken separately in two conical flasks and were made upto 5 ml by

adding 0 1 M phosphate buffer Five ml of two per cent sterilized agar maintained at 42 C was added to cell suspension kept in first flask whereas five ml of two per cent sterilized sodium alginate was added to cell suspension kept in second flask Both the samples were mixed using a vortex mixer and they were kept at 42 C in a water bath for two to three minutes and added to chilled toluene dropwise using a 10 ml cream pipette so as to form agar beads of uniform size of five mm in diameter These beads were allowed to cure for 15 to 30 minutes in toluene before it was removed and washed with normal saline 10 to 15 times in order to remove toluene The beads were then packed in sterilized glass column having 50 cm length and five cm diameter Two such separate columns, each containing 10 g immobilized cells were prepared and used for hydrolysing the lactose content in milk and whey After each trial these columns were washed thoroughly with distilled water and stored in cold room maintained at 4 C when it was not in use

3.3 Preparation and condensation of cottage cheese whey

Fresh good quality cow milk was received from KAU Dairy Plant, Mannuthy pasteurized by a batch pasteurizer and cooled to 30 C The rennet, (Rennilase 150 L - Novo Nordisk) diluted to 1 4 was added to milk at a rate of four ml per litre The

enzyme was thoroughly mixed with milk and kept undisturbed till a firm coagulum was obtained

The coagulum so formed was cut with the help of cheese knives first horizontally and then vertically to get smaller cubes of uniform size The temperature of the curd was then raised slowly to 40 C and left for five minutes before drainage of whey The whey was then drained out after filtering through a muslin cloth The collected whey was heated to 100 C for 10 minutes and again filtered so that the product obtained was free from residual rennet as well as precipitated proteins

The cheese whey was then condensed approximately to 2 1 concentration using a vacuum condensor ('Anhydro type Lab E W O, 1688") at 50 C with a vacuum of 76 cm of mercury Slightly over condensed whey so obtained was standardised to get 12 per cent total solids by adding sufficient quantity of distilled water

3.4 Lactose hydrolysis

The hydrolysis of lactose present in milk/whey was carried out by batch method using both of the immobilized enzyme system and the efficiency of both the systems were compared by estimating the percentage of lactose hydrolysis at fixed time intervals

Two hundred and fifty millilitre of skim milk/whey was allowed to react properly with the enzyme systems separately by mixing at every 15 minute intervals The reaction was allowed to carry out at 30 C and the samples were collected at every 30 minute intervals to estimate the rate of lactose hydrolysis After holding the samples for four hours within the first column, prepared with agar as immobilizing agent, the desired percentage (50 per cent) of lactose hydrolysis was achieved Whereas with the second column prepared using sodium alginate took more time for hydrolysing the same sample upto 50 per cent level Since the efficiency of agar was found to be better than sodium alginate, it was selected for further studies

For estimating the lactose in the samples, the procedure described by Nickerson *et al* (1975) was followed

3.4 1 Estimation of lactose Reagents

a Zinc acetate phosphotungstic acid (ZAPT) reagent

Prepared by dissolving 25 g zinc acetate and 12 5 g phosphotungstic acid in water Then 20 ml of glacial acetic acid was added and this was diluted to 100 ml

b Glycine - sodium hydroxide buffer

By mixing 150 ml of glycine solution containing 2 4768 g glycine and 1 935 g sodium chloride with 850 ml of 0 385 N sodium hydroxide (pH-12 8)

c Methylamine solution

Five per cent of methylamine hydrochloride in distilled water, which was stored in refrigerator

d Sodium sulfite solution

Freshly prepared by dissolving one g of sodium sulfite in distilled water and diluting to 100 ml

e Lactose standard solutions

(i) Stock solution Prepared by dissolving 2 6315 g of lactose monohydrate USP grade and diluting to 200 ml with 0 1 per cent Benzoic acid stored in refrigerator

(ii) Working solutions with 0 5, 0 75, 1 00, 1 25 and 1 5 mg lactose/ml were prepared by diluting 10 15, 20, 25 and 30 ml stock solutions to 250 ml respectively, using distilled water

Preparation of sample

a To eight ml of whey/milk added one ml of ZAPT reagent diluted to 10 ml and mixed After 10 minutes it was filtered through whatman No 1 filter paper (corrected for volume of fat and proteins using the formula given by Grimbley, 1956)

$$\text{Corrected reading} = \text{Observed reading} \times 100 \quad \frac{(Px0\ 84+Fx1\ 07)}{100}$$

where P and F are the percentage of protein and fat in the sample

b To 0 5 ml filtrate, added 0 5 ml 1 N NaOH, diluted to 10 ml and filtered

c Diluted five ml of filtrate to 10 ml using distilled water which formed the sample

Procedure

Pipetted 5 ml each of standard (standard lactose solution) unknown (samples of milk/whey) and blank (distilled water) into 25 ml test tubes To this the following solutions were added

a Five ml glycine - sodium hydroxide buffer

b 0 5 ml methylamine solution

c 0 5 ml sodium sulfite solution and mixed thoroughly

d Heated the tubes in a thermostatically controlled water bath at 65°C for exactly 25 minutes and cooled immediately in ice water bath for two minutes to stop the reaction

e Read absorbance against blank at 540 nm in spectronic-20 spectrophotometer Lactose concentration was then obtained from standard curve

A standard curve was prepared by plotting concentration of lactose solution in x-axis and corresponding absorbance in y-axis Lactose concentration ranging from 0 5 mg per ml to 1 5 mg per ml was used for preparing standard curve

3.5 Culture maintenance in different media

Four different media were selected to maintain the selected strains of lactic acid bacteria The media included

(i) Skim milk (M_1)

(ii) 50 per cent lactose hydrolyzed skim milk - (M_2)

(iii) Condensed whey with 12 per cent total solids - (M_3) and

(iv) 50 per cent lactose hydrolysed condensed whey (M_4)

Condensed whey and 50 per cent lactose hydrolysed condensed whey (M_3 and M_4) with a total solid content of 12 per cent was fortified with cysteine (0 05 per cent) and yeast extract (0 25 per cent), and the media were sterilized by autoclaving at 115 C at 0 70 kg/cm^2 pressure for 30 minutes

The skim milk and lactose hydrolysed skim milk were sterilized by autoclaving at 121 C and 1 05 kg/cm^2 pressure for 15 minutes, before inoculating with the following strains of lactic acid bacteria

(i) *S thermophilus*
(ii) *L delbrueckii* sub sp *bulgaricus*
(iii) *L lactis*
(iv) *L acidophilus* and
(v) *B bifidum*

After inoculating the media with two per cent level of cultures and incubating for 18 hours at their optimum temperatures, the cultures were tested for their

(i) titratable acidity
(ii) pH and
(iii) total count

3 5 1 Titratable acidity

Titratable acidity of the cultures were measured as per Indian standards, IS 1479 part II (1961) by titrating the sample against 0 1 N sodium hydroxide using one per cent phenolphthalein as indicator

3.5 2 pH

pH of the samples were monitored by an electronic digital pH meter

3.5 3 Total count

Direct microscopic count was used to find out total count in the samples After diluting the samples to 1 10, 0 01 ml of the sample was spread over the outlined area of one centimeter square (100 mm^2) on a grease free microscopic slide with the help of a Breeds pipette After making a uniform smear, it was air dried and stained with Newman's stain and then examined under the microscope The number of organisms per field was counted and average number per field was determined after examining 15 fields Total number of organism per ml was then calculated by multiplying the average number of organisms per field by the microscopic factor

For determining the microscopic factor (MF) the following formula was used

$$MF = \frac{100 \times 100 \times 10}{\pi r^2}$$

where r = Radius of microscopic field

The average number of organisms per field multiplied by the MF yielded the number of organisms per millilitre of sample

3.6 Preparation of low lactose fermented milk products

3 6 1 Yogurt

Fresh good quality cow milk was obtained from University Dairy Plant, Mannuthy The fat content of the milk was standardised to 3 5 per cent This sample was divided into two equal parts of 500 ml each and used for the preparation of treatment and control yogurt

The first half of the milk was subjected to 50 per cent lactose hydrolysis by passing through immobilized cell column and used for the preparation of treatment yogurts (Y_1 and Y_2) For preparing control yogurt (Y_3 and Y_4) ordinary milk standardised to 3 5 per cent fat was used

After prewarming and filtration both the samples (lactose hydrolysed and ordinary milk) were standardised to

3 5 per cent fat and 16 per cent solids-not-fat by adding sufficient quantity of skim milk powder The fortified milk samples were then heated to 60°C and homogenized at 2000 to 2500 psi The samples were then heat treated at 85°C for 30 minutes and cooled to 30°C to obtain two types of basic yogurt mixes

The yogurt mixes after cooling, were further divided into equal parts of 250 ml each for preparing two treatment and two control products

Among the media M_1, M_2, M_3 and M_4 the performance of the cultures were found to be better in M_2 and M_4 So the cultures maintained in media M_2 and M_4 were selected for further steps

Depending upon the type of yogurt mix and media used for culture maintenance, different products were named as Y_1, Y_2, Y_3 and Y_4

Y_1 - Where 50 per cent lactose hydrolysed milk and cultures maintained in 50 per cent lactose hydrolysed skim milk (M_2) was used

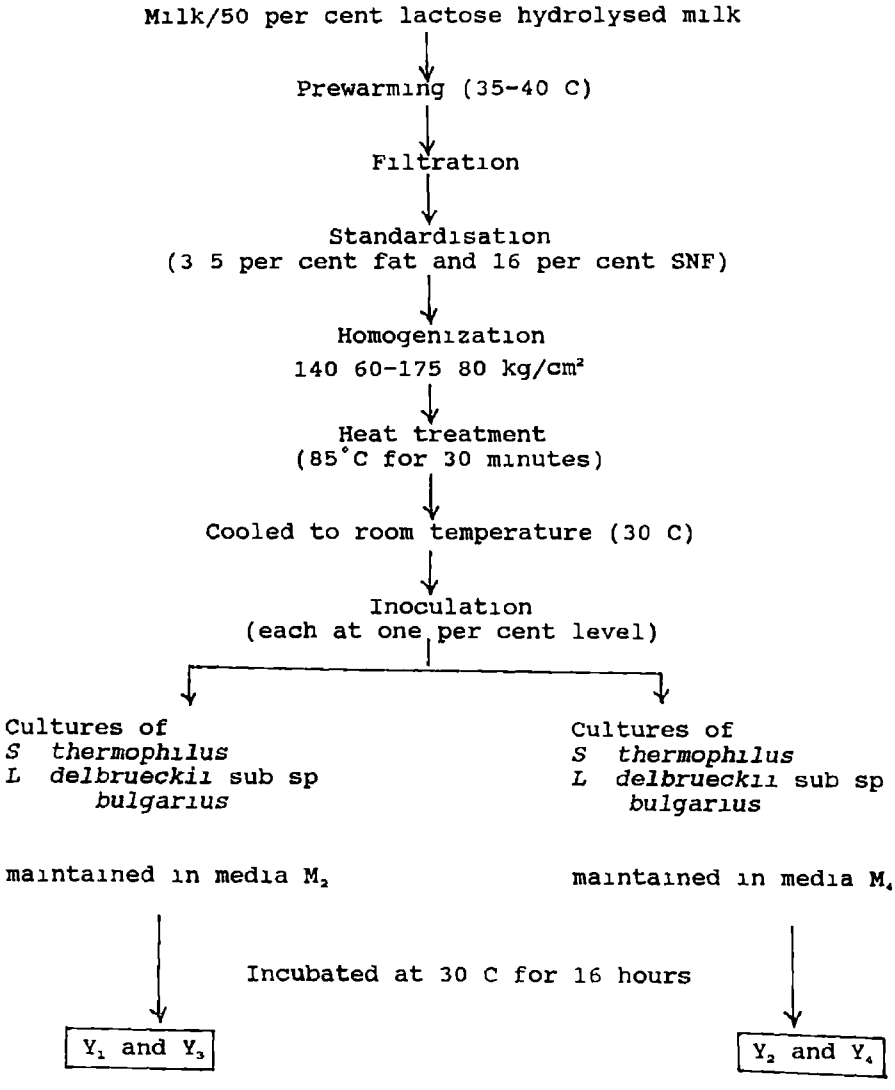
Y_2 - Where 50 per cent lactose hydrolysed milk and cultures maintained in 50 per cent lactose hydrolysed whey (M_4) was used

Y_3 - Ordinary milk and cultures maintained in 50 per cent lactose hydrolysed skim milk (M_2) was used

Y_4 - Ordinary milk and cultures maintained in 50 per cent lactose hydrolysed whey (M_4) was used

Active cultures of *S thermophilus* and *L delbrueckii* sub sp *bulgaricus* maintained in media M_2 and M_4 were inoculated into basic yogurt mixes at one per cent level each and incubated at room temperature for 16 hours, in order to obtain four different types of yogurt samples Y_1 Y_2 Y_3 and Y_4

Flow diagram for manufacture of yogurt



Y_1 - Yogurt prepared with 50 per cent lactose hydrolysed milk and cultures maintained in 50 per cent lactose hydrolysed skim milk

Y_2 - Yogurt prepared with 50 per cent lactose hydrolysed milk and cultures maintained in 50 per cent lactose hydrolysed whey

Y_3 - Yogurt prepared with ordinary milk and cultures maintained in 50 per cent lactose hydrolysed skim milk

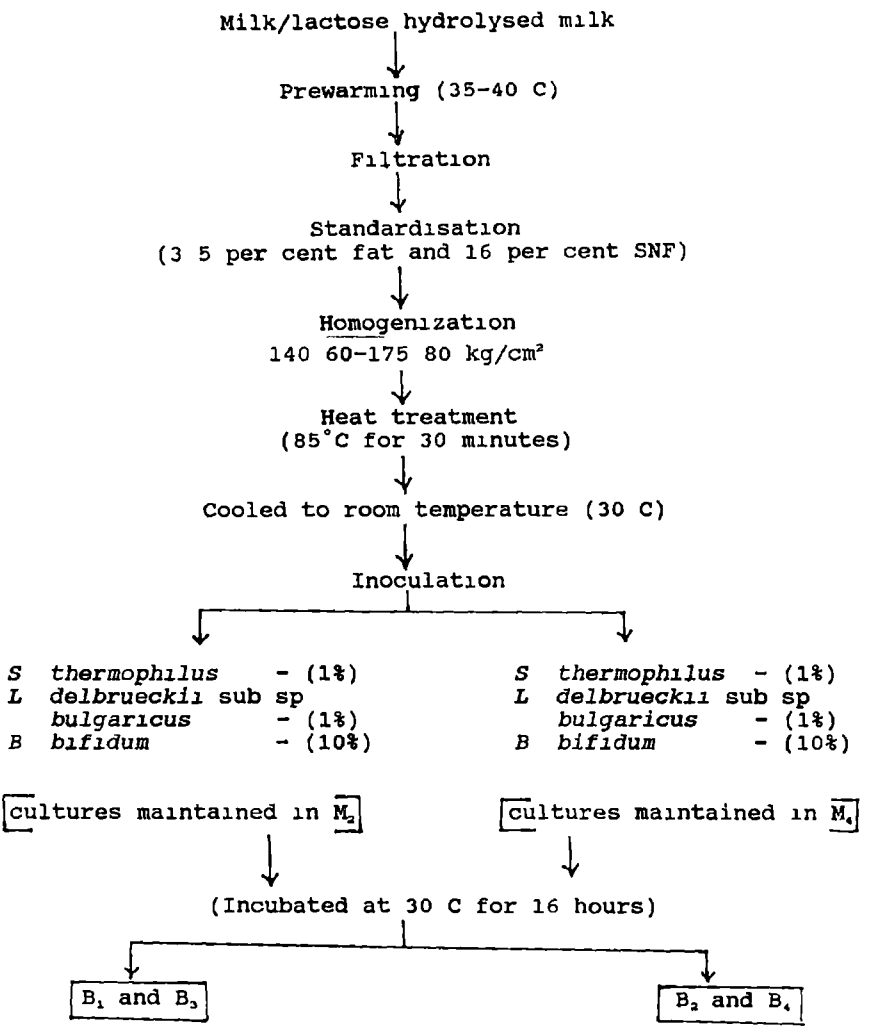
Y_4 - Yogurt prepared with ordinary milk and cultures maintained in 50 per cent lactose hydrolysed whey

3 6 2 Bifidus yogurt

The same method of preparation of basic yogurt mix was followed for Bifidus yogurt also The four different types of Bifidus yogurt samples were named as B_1, B_2 B_3 and B_4 depending on the type of yogurt mix and the media used for culture maintenance

The four different types of yogurt mixes were inoculated with active cultures of *S thermophilus L delbrueckii* sub sp *bulgaricus* and *B bifidum* maintained separately in media M_2 and M_4, at a ratio of 1 1 10 and incubated at room temperature for 16 hours The different Bifidus yogurt samples prepared were stored at 4 C until further analysis

Flow diagram for manufacture of Bifidus yogurt



B - Bifidus yogurt prepared with 50 per cent lactose hydrolysed milk and cultures maintained in 50 per cent lactose hydrolysed skim milk

B_2 - Bifidus yogurt prepared with 50 per cent lactose hydrolysed milk and cultures maintained in 50 per cent lactose hydrolysed whey

B_3 - Bifidus yogurt prepared with ordinary milk and cultures maintained in 50 per cent lactose hydrolysed skim milk

B_4 - Bifidus yogurt prepared with ordinary milk and cultures maintained in 50 per cent lactose hydrolysed whey

3 6 3 Acidophilus milk

Fresh, good quality milk after collecting from KAU Dairy Plant, the fat content was standardised to 3 5 per cent The milk was then divided into two equal parts of 500 ml each and first half of the milk was subjected to 50 per cent lactose hydrolysis by passing through immobilized cell column and used for preparing treatment products The other half of milk was used as such for control product

After prewarming filtration and homogenization of milk samples at 2000 to 2500 psi heat treated the samples at 85 C for 30 minutes After cooling to 37 C the samples were divided into two equal parts of 250 ml each and separately

inoculated with cultures of *L acidophilus* at one per cent level, maintained in media M_2 and M_4

The description of acidophilus milk under different treatments are given below

A_1 - Acidophilus milk prepared with 50 per cent lactose hydrolysed milk and culture maintained in 50 per cent lactose hydrolysed skim milk (M_2)

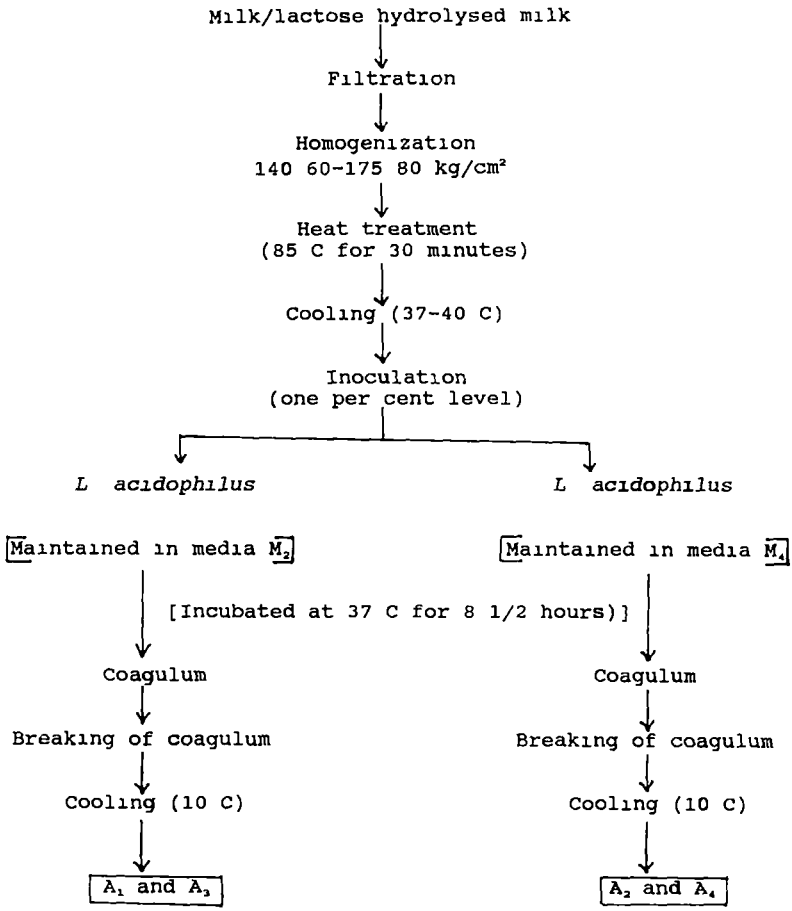
A_2 - Acidophilus milk prepared with 50 per cent lactose hydrolysed milk and culture maintained in 50 per cent lactose hydrolysed whey (M_4)

A_3 - Product prepared with ordinary milk and culture maintained in media (M_2)

A_4 - Product prepared with ordinary milk and culture maintained in media (M_4)

After inoculating the milk samples with *L acidophilus* incubated at 37°C for 8 1/2 hours and after developing acidity at one per cent level, the coagulum was broken Transferred the product to retail containers and stored at refrigeration temperature until it was used for further analysis

Flow diagram for manufacture of Acidophilus milk



A_1 - Acidophilus milk prepared with 50 per cent lactose hydrolysed milk and cultures maintained in 50 per cent lactose hydrolysed skim milk

A_2 - Acidophilus milk prepared with 50 per cent lactose hydrolysed milk and cultures maintained in 50 per cent lactose hydrolysed whey

A_3 - Acidophilus milk prepared with ordinary milk and cultures maintained in 50 per cent lactose hydrolysed skim milk

A_4 - Acidophilus milk prepared with ordinary milk and cultures maintained in 50 per cent lactose hydrolysed whey

3.7 Analysis of the products

All the three products, under four different treatments (Y Y_2 Y_3, Y_4 B_1, B_2 B_3, B_4 and A_1 A_2 A_3, A_4) were subjected to biochemical and microbiological analysis

3 7 1 Biochemical analysis

All the three products under different treatments were subjected to analysis for the following biochemical parameters

3 7 1 1 Acidity

Titratable acidity of all the products were measured as per the method described by Indian standards IS 1479 a part II (1961)

3 7 1 2 pH

An electronic digital pH meter was used to estimate the pH of the samples

3 7 1 3 Tyrosine value

The proteolysis was determined by estimating free tyrosine according to the method of Lowry *et al* (1951)

Reagents

(i) 12 per cent Trichloro acetic acid

Prepared by dissolving 12 g Trichloro acetic acid in 100 ml distilled water

(ii) Two per cent sodium carbonate in 0 1 N sodium hydroxide

Prepared by dissolving two gram sodium carbonate in 100 ml 0 1 N sodium hydroxidse (Reagent I)

(iii) One per cent sodium potassium tartrate

Prepared by dissolving one gram sodium potassium tartrate in 100 ml distilled water (Reagent II)

(iv) 0 5 per cent copper sulphate solution

Prepared by dissolving 0 5 g copper sulphate in 100 ml distilled water (Reagent III)

(v) Alkaline copper reagent was prepared by mixing 48 ml of Reagent I with one ml each of reagent II and III

(vi) Diluted Folin reagent (Folin reagent was diluted with equal volume of distilled water)

(vii) Standard tyrosine solutions

L tyrosine was dissolving in minimum quantity of 0 1 N sodium hydroxide solution and the final concentration was adjusted to 0 1 mg per ml using distilled water

Procedure

To five gram of the yogurt sample equal amount of 12 per cent Trichloro acetic acid was added After vigorous shaking it was allowed to stand for 15 minutes and then filtered using Whatman No 42 filter paper

To one ml of the filtrate five ml alkaline copper reagent was added, contents were mixed well and incubated at 37 C for 10 minutes in a water bath This was followed by addition of 0 5 ml Folin's reagent mixed well and again incubated at 37 C for 20 minutes in the same water bath At the end of incubation, the blue colour developed was measured at 660 nm using a spectronic-20 A blank was made using distilled water A standard curve was prepared by dissolving 10 mg L tyrosine in minimum quantity of 0 1 N NaOH and final volume was made up with distilled water to 0 1 mg/ml Proteolytic activity was expressed as milligram of Tyrosine liberated per gram of sample

3 7 2 Microbiological analysis

3 7 2 1 Preparation of diluents

One gram of sample was transferred aseptically into nine ml of sterile normal saline Serial dilutions were prepared upto 10^{9} One millilitre each of sample from dilutions 10^{6} to 10^{9} were used for estimating the total viable counts

3 7 2 2 Enumeration of *S thermophilus* and *L delbrueckii* sub sp *bulgaricus* in yogurt and Bifidus yogurt

Yogurt lactic agar (Matalon and Sandine, 1986) was used for enumeration of *S thermophilus* and *L delbrueckii* sub sp *bulgaricus* counts One millilitre samples each from dilutions of 10^{6} to 10^{9} were transferred into sterile petriplates in duplicate and melted yogurt lactic agar medium approximately 15 ml quantity was then poured and mixed thoroughly by rotating the petridishes After solidification of the media, the petri plates were placed upside down in an incubator at 37 C

The composition of yogurt lactic agar was as follows

Tryptone	-	20 g
Yeast extract	-	5 g
Gelatin	-	2 g
Glucose	-	5 g
Sucrose	-	5 g
Lactose	-	5 g
Sodium chloride	-	4 g
Sodium acetate	-	1 5 g
Ascorbic acid	-	0 5 g
Agar	-	15 g
Distilled water	-	1000 ml

The medium was supplemented with 0 1 per cent Tween 80 and gently heated with continuous stirring to dissolve thoroughly all the ingredients after which pH was adjusted to 6 8 Agar was then added at 1 5 per cent level and the medium was autoclaved at 121 C for 15 minutes at 15 lbs pressure

According to the differential colony characteristics described by Matalon and Sandine (1986) the colonies of *S thermophilus* and *L delbrueckii* sub sp *bulgaricus* were enumerated separately

Small white, diagonal colonies without cloudy zone indicated *S thermophilus*, whereas *L delbrueckii* sub sp *bulgaricus* colonies appeared as round and smooth with a clear zone around

3 7 2 3 Enumeration of *B bifidum*

For enumeration of *B bifidum* in yogurt lithium chloride sodium propionate agar (LP agar) was used (Lapierre *et al* , 1992)

The composition of LP agar was as follows

Liver infusion	-	35 g
Lactose	-	10 g
Peptone		10 g
Sodium chloride	-	2 g

Lithium chloride	-	2 g
Sodium propionate	-	3 g
Agar	-	20 g
Distilled water	-	1000 ml

All the ingredients of the medium were dissolved in distilled water and pH was adjusted to 6 7 ± 0 2 The medium was sterilized by autoclaving

Plates were incubated at 37 C for 48 hours under carbondioxide tension *B bifidum* appeared as white, smooth convex and glistening colonies

3 7 2 4 Enumeration of *L acidophilus*

Enumeration of *L acidophilus* was achieved by plating in MRS agar and incubating at 37 C for 24 hours

Colonies of *L acidophilus* appeared as white round and mucoid having two to three millimeter diameter

The composition of MRS agar was given below

Peptone	-	10 g
Beef extract	-	10 g
Yeast extract	-	5 g
Glucose	-	20 g
Tween 80	-	1 ml

Dipotassium monohydrogen phosphate	-	2 g
Sodium acetate	-	5 g
Triammonium citrate	-	2 g
Magnesium sulphate	-	0 2 g
Manganese sulphate	-	0 05 g
Agar	-	15 g
Distilled water	-	1000 ml

All the ingredients of the medium were dissolved in distilled water and the pH was adjusted to 6 8 The medium was sterilized at 121 C for 15 minutes at 15 lbs pressure

3 7 3 Sensory evaluation of products

All the three products under different treatments were evaluated for their sensory characteristics by a panel of five judges The score card proposed by Pearce and Heap (1974) for yogurt was adopted for evaluation of bifidus yogurt and acidophilus milk Average scores obtained from five members for each replication was used for statistical analysis

Score card proforma for evaluation of yogurt was as follows

Score card

Yogurt evaluation

Date
Taster
Code No

a appearance and colour defects

b body and texture defects

c flavours defects

Overall scores

Judged the three characteristics on 1-5 scale

5 Excellent
4 Very good
3 Good
2 Fair
1 Poor

The overall score is obtained by multiplying the flavour score by 2 and then adding the score to the rest

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

3.8 Statistical analysis

The data obtained from the experiment were tabulated and subjected to statistical analysis The method described by Snedecor and Cochran (1967) was followed for this Completely randomised design (CRD) was used for the analysis of data regarding acidity, pH and total count of different cultures maintained in four different media Titratable acidity pH tyrosine value, viable count and organoleptic scores for different products under different treatments were also subjected to statistical analysis using completely randomised design Acidity was analysed after angular transformation

Results

RESULTS

4.1 Development of an immobilized enzyme system

An efficient β-galactosidase enzyme system was developed using permeabilized cells *K fragilis* as an enzyme source and food grade agar as an immobilizing agent for reducing lactose content in milk which inturn was utilized for preparing fermented milk products with low lactose Possibilities of utilization of whey, a by-product, as a medium for culture maintenance was also explored A brief description of its procedure and the results obtained are given below

Three selected organisms viz, *S thermophilus*, *L delbrueckii* sub sp *bulgaricus* and *K fragilis* were screened for their β-galactosidase specific activity and *K fragilis* was selected as the best from among them Active cells of *K fragilis* were grown in lactose broth, harvested by centrifugation and were freeze dried One gram of freeze dried cells of *K fragilis* was dispensed in 100 ml 0 1 M phosphate buffer and the cell wall of the yeast was permeabilized by adding sufficient concentration of potassium sorbate After permeabilization potassium sorbate was removed by centrifugation and collected the cell mass These cells were used for the preparation of immobilized enzyme system and for lactose hydrolysis Suitability of sodium alginate as

well as agar as immobilizing agents were also screened in the present study

Two separate columns were prepared using *K fragilis* as enzyme source and sodium alginate/agar as immobilizing agent The hydrolysis of lactose present in skim milk and whey was carried out in batchwise using the above described immobilized systems The rate of lactose hydrolysis by both the columns were determined separately Since the efficiency of the system prepared using agar as immobilizing agent was found to be better, that system was selected for further studies

The results obtained regarding the β-galactosidase specific activity of different organisms efficiency of the system in terms of percentage of lactose hydrolysis within a fixed time interval and repeatability are given below

4 1 1 β-galactosidase specific activity of permeabilized cells

The β-galactosidase specific activity of permeabilized cells of *S thermophilus, L delbrueckii* sub sp *bulgaricus* and *K fragilis* were measured in terms of number of units One unit is defined as the number of moles of orthonitro phenol (ONP) released per millilitre per minute

The data pertaining to β-galactosidase specific activity of different organisms is shown in Table 1 From the table it

could be seen that the mean β-galactosidase specific activity of *S thermophilus* was 1 75 units ranging from 1 55 to 1 86 The mean value for *L delbrueckii* sub sp *bulgaricus* was 1 25 units and the values ranged from 1 15 to 1 35 units Whereas *K fragilis* cells showed the maximum specific activity of 2 46 units The values being ranged from 2 21 to 2 83 untis Since the β-galactosidase specific activity of *K fragilis* was found to be the highest, it was selected as the source of enzyme for further study

Permeabilized cells of *K fragilis* were immobilized separately with sodium alginate as well as agar and the data regarding the rate of lactose hydrolysis using both systems are shown in Table 2

From the table it could be seen that 50 per cent of lactose present in 250 ml of milk was get hydrolysed after holding in the system prepared with agar for four hours at 30 C, whereas only 45 per cent of hydrolysis was obtained with sodium alginate as immobilizing agent The rate of lactose hydrolysis was found to be the maximum within first half an hour with both the systems used and thereafter a decline in the rate of hydrolysis was observed Since the efficiency was found to be higher for the system prepared with agar as immobilizing agent that system was selected for further studies

Table 1 β-galactosidase specific activity of different cultures (units)

Replication	*S thermophilus*	*L delbrueckii* sub sp *bulgaricus*	*K fragilis*
1	1 86	1 21	2 61
2	1 75	1 15	2 83
3	1 85	1 28	2 30
4	1 55	1 35	2 21
Mean	1 75	1 25	2 46

Table 2 Rate of lactose hydrolysis obtained with two different immobilized enzyme systems (per cent)

Time intervals (h)	Sodium alginate		Agar	
	Lactose hydrolysis (%)	Remaining lactose (g)	Lactose hydrolysis (%)	Remaining lactose (g)
0	0	4 80	0	4 80
1/2	23	3 70	27	3 50
1	33	3 22	37	3 02
2	42	2 78	45	2 64
3	44	2 69	48	2 50
4	45	2 64	51	2 35

This system was repeatedly used in five batches without any change in its efficiency or mechanical stability of the beads but after fifth passing a reduction in activity was observed Due to long storage a slight brownish discolouration was also observed in the beads So a new column was prepared for hydrolysis of lactose for further steps

4.2 Performance of starter organisms in different media

Four different media viz skim milk (M_1), 50 per cent lactose hydrolysed skim milk (M_2) condensed whey (M_3) and 50 per cent lactose hydrolysed condensed whey (M_4) after proper sterilization and cooling were inoculated with the following selected strains of lactic acid bacteria namely, *S thermophilus*, *L delbrueckii* sub sp *bulgaricus*, *B bifidum*, *L lactis* and *L acidophilus* at two per cent level The performance of these cultures in different media were studied in terms of acidity pH and total count after incubating at their optimum temperature for 18 hours The results obtained are given below

4 2 1 Change in acidity

4 2 1 1 S thermophilus

The overall mean acidity developed by *S thermophilus* maintained in four different media is shown in Table 3a

Table 3a Acidity produced by *S thermophilus* in different media (per cent of lactic acid)

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	0 63	0 63	0 60	0 69
2	0 68	0 64	0 62	0 65
3	0 65	0 67	0 94	0 82
4	0 74	0 83	0 86	0 88
5	0 77	0 78	0 78	0 82
6	0 72	0 76	0 75	0 80
Mean	0 70	0 72	0 76	0 78
S E ±	0 02	0 03	0 05	0 04

Table 3b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 002	0 001	0 653 NS
Within treatments	20	0 016	0 001	
Total	23	0 018		

NS - Not significant

It could be seen from the Table that the mean acidity produced by *S thermophilus*, when maintained in skim milk (M_1), lactose hydrolysed skim milk (M_2), whey (M_3) and lactose hydrolysed whey (M_4) were 0 70 ± 0 02, 0 72 ± 0 03, 0 76 ± 0 05 and 0 78 ± 0 04 per cent of lactic acid respectively Eventhough a slight increase in acidity was observed when the culture was maintained in whey based media (M_3 and M_4), the increase was found to be statistically not significant (Table 3b)

4.2 1 2 L delbrueckii sub sp bulgaricus

The overall mean acidity produced by *L delbrueckii* sub sp *bulgaricus* in four different media is presented in Table 4a The mean lactic acid produced by *L delbrueckii* sub sp *bulgaricus* in media M_1 was 1 59 ± 0 07, in M_2 the value was 1 64 ± 0 07 and in M_3 and M_4 the values were 1 57 ± 0 08 and 1 61 ± 0 08 per cent of lactic acid respectively

From the table it could be seen that the performance of *L delbrueckii* sub sp *bulgaricus* was apparently better when the media was subjected to lactose hydrolysis In between the media M_2 and M_4 the acidity produced was found to be comparatively higher in M_2 (lactose hydrolysed milk) When the media M_1 and M_3 were compared, it was found that the acidity produced by *S thermophilus* was almost same in both However on statistical analysis no significant differences

Table 4a Acidity produced by *L delbrueckii* sub sp *bulgaricus* in different media (per cent of lactic acid)

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	1 52	1 60	1 21	1 29
2	1 45	1 49	1 44	1 44
3	1 35	1 38	1 65	1 71
4	1 72	1 78	1 84	1 81
5	1 73	1 80	1 68	1 76
6	1 79	1 79	1 62	1 64
Mean	1 59	1 64	1 57	1 61
S E ±	0 07	0 07	0 08	0 08

Table 4b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 001	0 000	0 357 NS
Within treatments	20	0 027	0 001	
Total	23	0 028		

NS - Not significant

were noticed in acidity among the cultures maintained in all these media (Table 4b)

4.2 1 3 *B bifidum*

The overall mean acidity developed by *B bifidum* in different media is presented in Table 5a From the table it could be seen that the overall mean acidity produced by *B bifidum* in media M_1, M_2, M_3 and M_4 were 1 20 ± 0 06 1 22 ± 0 07, 1 25 ± 0 11 and 1 28 ± 0 12 per cent of lactic acid respectively

The acidity produced by *B bifidum* was found to be comparatively higher in whey based media (M_3 and M_4) than skim milk media (M_1 and M_2) In between the media M_3 and M_4, the performance of the culture was found to be better in M_4 (lactose hydrolysed whey) However, statistical analysis revealed that there was no significant difference among them (Table 5b)

4.2.1 4 *L. Lactis*

The overall mean acidity produced by *L lactis* was found to be 0 72 ± 0 06, 0 73 ± 0 05 0 82 ± 0 07 and 0 85 ± 0 08 per cent of lactic acid respectively when grown in media M_1, M_2, M_3 and M_4 (Table 6a) From the Table it was observed that the performance of the culture was better in whey based media in comparison to skim milk

Table 5a Acidity produced by *B bifidum* in different media (per cent of lactic acid)

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	1 03	1 12	0 92	0 94
2	1 21	1 38	0 98	0 97
3	1 09	1 10	1 46	1 42
4	1 10	0 99	1 54	1 60
5	1 33	1 36	1 48	1 56
6	1 41	1 39	1 09	1 18
Mean	1 20	1 22	1 25	1 28
S E ±	0 06	0 07	0 11	0 12

Table 5b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 000	0 000	0 057 NS
Within treatments	20	0 037	0 002	
Total	23	0 037		

NS - Not significant

Table 6a Acidity produced by *L lactis* in different media (per cent of lactic acid)

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	0 63	0 64	0 62	0 68
2	0 62	0 63	0 78	0 77
3	0 88	0 77	0 71	0 72
4	0 57	0 69	0 86	0 86
5	0 72	0 86	1 10	1 20
6	0 86	0 88	0 87	0 89
Mean	0 72	0 73	0 82	0 85
S E ±	0 06	0 05	0 07	0 08

Table 6b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 010	0 003	1 294 NS
Within treatments	20	0 052	0 003	
Total	23	0 062		

NS - Not significant

The results showed that in 50 per cent lactose hydrolysed media the culture developed more acidity In between media M and M_2 (skim milk media) the performance of the culture was found to be comparatively better in M_2 (lactose hydrolysed milk) But analysis of variance revealed there was no significant difference among these values (Table 6b)

4 2 1 5 *L Acidophilus*

The overall mean acidity of *L acidophilus* under different treatments are given in Table 7a The acidity produced by *L acidophilus* was same when it was maintained in media M_1 and M_2 and the values were around 1 66 per cent of lactic acid In media M_3, the lowest value for acidity (1 55) was observed In lactose hydrolysed whey media (M_4), a value of 1 61 was noted

From these values it was seen that the performance of *L acidophilus* was better in skim milk media (M_1 and M_2) when compared to whey based media (M_3 and M_4) However the difference was found to be not significant when statistical analysis was done (Table 7b)

4 2 2 Change in pH

4 2 2 1 S thermophilus

The overall mean pH values for *S thermophilus* in different media are given in Table 8a

Table 7a Acidity produced by *L acidophilus* in different media (per cent of lactic acid)

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	1 71	1 57	1 43	1 41
2	1 25	1 32	1 24	1 28
3	1 98	1 98	1 82	1 73
4	1 88	1 89	1 61	1 87
5	1 68	1 70	1 69	1 84
6	1 47	1 52	1 55	1 58
Mean	1 66	1 66	1 55	1 61
S E ±	0 11	0 10	0 10	0 10

Table 7b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 000	0 000	0 090 NS
Within treatments	20	0 019	0 001	
Total	23	0 019		

NS - Not significant

Table 8a pH of *S thermophilus* in different media

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	4 27	4 31	4 20	4 16
2	4 32	4 49	4 09	4 11
3	4 28	4 30	4 24	4 21
4	4 74	4 35	4 63	4 42
5	4 47	4 44	4 56	4 69
6	4 38	4 28	4 28	4 24
Mean	4 41	4 35	4 33	4 31
S E ±	0 07	0 04	0 08	0 09

Table 8b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 035	0 012	0 355 NS
Within treatments	20	0 666	0 033	
Total	23	0 701		

NS - Not significant

From the Table it could be seen that the mean pH values of *S thermophilus* for treatments M_1 M_2 M_3 and M_4 were 4 41 ± 0 07 4 35 ± 0 04 4 33 ± 0 08 and 4 31 ± 0 09 respectively On statistical analysis no significant difference was noted among these values (Table 8b)

4 2 2 2 *L. delbrueckii* sub sp *bulgaricus*

The overall mean pH values obtained for *L delbrueckii* sub sp *bulgaricus* under different treatments are given in Table 9a From the Table the mean value was found to range from 3 52 to 3 60 indicating that there was no statistical difference among them (Table 9b)

4 2 2 3 *B bifidum*

The pH values for *B bifidum* under different treatments and their overall mean values are presented in Table 10a The overall mean pH values obtained were 3 61 ± 0 06, 3 51 ± 0 07 3 46 ± 0 05 and 3 42 ± 0 03 respectively when the culture was maintained in media M_1 M_2 M_3 and M_4 Comparison of treatment means revealed that there was no significant difference among these pH values (Table 10b)

4 2 2 4 *L lactis*

The overall mean pH value obtained when the culture maintained in four different media viz M_1 M_2 M_3 and M_4 were

Table 9a pH of *L delbrueckii* sub sp *bulgaricus* in different media

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	3 49	3 36	3 51	3 44
2	3 60	3 56	3 61	3 60
3	3 59	3 57	3 66	3 63
4	3 43	3 46	3 65	3 58
5	3 48	3 54	3 55	3 42
6	3 52	3 65	3 63	33 64
Mean	3 52	3 52	3 60	3 55
S E ±	0 03	0 04	0 02	0 04

Table 9b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 026	0 009	1 279 NS
Within treatments	20	0 137	0 007	
Total	23	0 163		

NS - Not significant

Table 10a pH of *B bifidum* in different media

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	3 43	3 41	3 46	3 45
2	3 56	3 36	3 39	3 39
3	3 78	3 42	3 32	3 31
4	3 76	3 79	3 69	3 53
5	3 68	3 58	3 50	3 49
6	3 47	3 49	3 40	3 36
Mean	3 61	3 51	3 46	3 42
S E ±	0 06	0 07	0 05	0 03

Table 10b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 124	0 041	2 346 NS
Within treatments	20	0 352	0 018	
Total	23	0 476		

NS - Not significant

Table 11a pH of *L lactis* in different media

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	4 34	4 33	4 31	4 04
2	4 09	4 28	4 25	4 28
3	4 30	4 42	4 28	4 02
4	4 52	4 23	4 20	4 14
5	4 43	4 01	3 95	4 24
6	4 38	4 25	4 21	4 33
Mean	4 35	4 25	4 20	4 18
S E ±	0 08	0 06	0 05	0 05

Table 11b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 118	0 039	1 284 NS
Within treatments	20	0 611	0 031	
Total	23	0 729		

NS - Not significant

4 35 ± 0 08, 4 25 ± 0 06 4 21 ± 0 05 and 4 18 ± 0 05 respectively (Table 11a) From the table it could be seen that the pH value of *S thermophilus* was comparatively lower when the culture was maintained in M_4 (lactose hydrolysed whey) and the highest pH value was observed in media M_1 (4 35) However all the values were found to be not significant statistically (Table 11b)

4 2 2 5 *L acidophilus*

The mean values of pH obtained for *L acidophilus* is presented in Table 12a Eventhough the lowest pH value of 3 40 was observed in M_1 and highest value of 3 55 was observed in M_3 on statistical analysis these difference in values were found to be not significant (Table 12b)

4 2 3 Total count

4 2 3 1 *S thermophilus*

The overall mean count of *S thermophilus* under treatment M was $4\ 11x10^8 \pm 0\ 42x10^8$ cfu/ml For treatments M_2 and M_3 the counts were $4\ 18x10^8 \pm 0\ 41x10^8$ and $4\ 24x10^8 \pm 0\ 57x10^8$ cfu/ml respectively In M_4 the count was $4\ 34x10^8 \pm 0\ 64x10^8$ cfu/ml (Table 13a)

From the Table it could be seen that among the four different media the media M_3 and M apparently stimulated the

Table 12a pH of *L acidophilus* in different media

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	3 46	3 40	3 50	3 47
2	3 66	3 33	3 41	3 71
3	3 48	3 42	3 56	3 48
4	3 33	3 40	3 51	3 47
5	3 21	3 48	3 82	3 29
6	3 23	3 44	3 48	3 45
Mean	3 40	3 41	3 55	3 45
S E ±	0 07	0 02	0 06	0 07

Table 12b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 083	0 028	1 425 NS
Within treatments	20	0 389	0 019	
Total	23	0 472		

NS - Not significant

Table 13a Total count of *S thermophilus* in different media ($x10^8$ cfu/ml)

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	4 72	3 97	2 48	2 73
2	2 88	3 73	2 98	2 88
3	3 73	3 98	4 77	6 92
4	4 37	4 47	6 21	5 24
5	5 71	5 96	5 14	4 09
6	3 23	2 98	3 86	4 21
Mean	4 11	4 18	4 24	4 34
S E ±	0 42	0 41	0 57	0 64

Table 13b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 0003	0 000	0 007 NS
Within treatments	20	0 342	0 017	

NS - Not significant

growth of *S thermophilus* In skim milk media (M and M_2) the growth of the culture was comparatively low However statistical analysis showed that there was no significant difference among the four media (Table 13b)

4 2 3 2 *L delbrueckii* sub sp *bulgaricus*

The overall mean total count of *L delbrueckii* sub sp *bulgaricus* when maintained in media M_1 M_2, M_3 and M_4 were $5\ 18x10^8 \pm 0\ 42x10^8$, $6\ 16x10^8 \pm 0\ 61x10^8$ $4\ 76x10^8 \pm 0\ 37x10^8$ and $5\ 21x10^8 \pm 0\ 27x10^8$ cfu/ml respectively (Table 14a)

From the table it could be seen that the number of *L delbrueckii* sub sp *bulgaricus* was higher when allowed to grow in 50 per cent lactose hydrolysed media Between skim milk and whey, skim milk was found to be better for their growth However, on statistical analysis it was found that, these differences in values were not significant (Table 14b)

4 2 3 3 *B. bifidum*

The overall mean total counts of *B bifidum* in different media are given in Table 15a

The values for total counts were $4\ 14x10^8 \pm 0\ 90x10^8$, $4\ 26x10^8 \pm 0\ 42x10^8$, $4\ 67x10^8 \pm 0\ 35x10^8$ and $4\ 67x10^8 \pm 0\ 44x10^8$ cfu/ml respectively, when culture was grown in media M_1, M_2, M_3 and M_4 (Table 15a) The performance of the culture in whey

Table 14a Total count of *L delbrueckii* sub sp *bulgaricus* in different media (x10^8 cfu/ml)

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	6 96	6 95	4 39	5 69
2	5 36	6 50	3 89	4 12
3	5 56	5 31	5 14	5 48
4	4 07	5 71	6 48	6 06
5	4 87	8 44	4 26	5 11
6	4 27	4 07	4 39	4 83
Mean	5 18	6 16	4 76	5 21
S E ±	0 42	0 61	0 37	0 27

Table 14b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 037	0 013	1 762 NS
Within treatments	20	0 143	0 007	

NS - Not significant

Table 15a Total count of *B bifidum* in different media ($x10^6$ cfu/ml)

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	2 09	4 27	4 33	4 26
2	6 37	5 66	3 48	4 21
3	3 36	3 87	5 96	6 48
4	1 24	2 58	5 24	5 96
5	5 56	4 18	4 32	4 14
6	6 21	5 60	4 68	3 96
Mean	4 14	4 26	4 67	4 84
S E ±	0 90	0 42	0 35	0 44

Table 15b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 060	0 020	0 716 NS
Within treatments	20	0 566	0 028	

NS - Not significant

based media was found to be better It showed a better growth than control (milk and skim milk) though the difference was not significant statistically when analysed

4 2 3 4 *L lactis*

The overall mean counts of *L lactis* in different media is presented in Table 16a

The mean counts of *L lactis* under different treatments M_1 M_2 M_3 and M_4 were $5.50 \times 10^8 \pm 0.75 \times 10^8$ $5.55 \times 10^8 \pm 0.60 \times 10^8$ $5.50 \times 10^8 \pm 0.59 \times 10^8$ and $5.58 \times 10^8 \pm 0.67 \times 10^8$ cfu/ml respectively Eventhough a slight increase in count was observed in lactose hydrolysed media (M_2 and M_4) on statistical analysis the difference was found to be not significant (Table 16b)

4 2 3 5 *L acidophilus*

The overall mean total counts of *L acidophilus* in different media are presented in Table 17a From the Table it could be seen that the overall mean acidophilus counts were $54.05 \times 10^8 \pm 4.15 \times 10^8$ $55.78 \times 10^8 \pm 4.79 \times 10^8$ $10.96 \times 10^8 \pm 2.70 \times 10^8$ and $12.99 \times 10^8 \pm 3.56 \times 10^8$ cfu/ml respectively for media M_1 M_2 M_3 and M_4

Pairwise comparison between lactose hydrolysed and ordinary samples showed that there was no significant

Table 16a Total count of *L lactis* in different media ($x10^8$ cfu/ml)

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	3 73	3 87	3 11	3 28
2	3 77	4 27	3 33	3 96
3	4 37	5 38	8 64	6 96
4	7 35	7 20	5 67	5 49
5	7 90	7 45	5 33	6 42
6	6 85	5 11	6 97	7 32
Mean	5 50	5 55	5 50	5 58
S E ±	0 75	0 60	0 59	0 67

Table 16b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 023	0 007	0 305 NS
Within treatments	20	0 509	0 025	

NS - Not significant

Table 17a Total count of *L acidophilus* in different media ($x10^6$ cfu/ml)

Replication	Treatment			
	M_1	M_2	M_3	M_4
1	48 70	38 80	8 68	7 96
2	39 70	55 60	5 87	6 48
3	67 00	74 50	23 60	26 80
4	61 10	59 60	12 80	21 10
5	59 60	56 40	8 38	8 77
6	48 20	49 80	6 44	6 83
Mean	54 05	55 78	10 96	12 99
S E ±	4 15	4 79	2 71	3 56

Table 17b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	3 108	1 036	30 35 **
Within treatments	20	0 682	0 034	

** - Highly significant

difference between M_1 and M_2 as well as between M_3 and M_4 But a highly significant difference was noted between whey based media and skim milk media, ie between M_1 and M_3 M_2 and M_3 M_1 and M_4 and M_2 and M_4 (Table 17b)

4.3 Biochemical and microbiological qualities of low lactose fermented milk products

Three types of fermented products viz yogurt bifidus yogurt and acidophilus milk were prepared with 50 per cent lactose hydrolysed milk and unhydrolysed milk, using cultures maintained in media M_2 and M_4 which were found to be better among the four media screened All the three products under different treatments were tested for their (i) acidity (ii) pH (iii) tyrosine value (iv) total lactic count and (v) sensory characteristics The results are given below

4 3 1 Yogurt

4 3 1 1 Acidity and pH

The overall mean values for acidity of yogurt under four different treatments are presented in Table 18a

The mean values obtained for treatment Y_1, Y_2, Y_3 and Y_4 were 1 13 $\pm$ 0 05 1 10 $\pm$ 0 04 1 08 $\pm$ 0 09 and 1 00 $\pm$ 0 04 per cent of lactic acid respectively A slightly higher level of acidity was observed for yogurt samples prepared with lactose hydrolysed milk (Y_1 and Y_2) when compared to control

Table 18a Acidity of yogurt samples under different treatments (per cent of lactic acid)

Replication	Treatment			
	Y_1	Y_2	Y_3	Y_4
1	1 30	1 04	1 34	1 02
2	1 17	1 25	1 03	0 99
3	1 23	1 17	1 20	1 18
4	0 95	0 95	0 83	0 86
5	1 06	1 12	0 86	0 99
6	1 06	1 09	1 22	0 98
Mean	1 13	1 10	1 08	1 00
S E ±	0 05	0 04	0 09	0 04

Table 18b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 003	0 001	1 393 NS
Within treatments	20	0 017	0 001	
Total	23	0 020		

NS Not significant

Table 19a pH of yogurt samples under different treatments

Replication	Treatment			
	Y_1	Y_2	Y_3	Y_4
1	4 21	4 15	3 91	4 24
2	4 08	4 03	4 04	4 00
3	4 32	4 68	4 74	4 77
4	4 52	4 62	4 83	4 27
5	4 02	4 01	4 00	4 02
6	4 10	4 03	4 06	4 15
Mean	4 20	4 24	4 26	4 27
S E ±	0 13	0 15	0 17	0 11

Table 19b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 015	0 005	0 041 NS
Within treatments	20	2 443	0 122	
Total	23	2 458		

NS - Not significant

samples (Y_3 and Y_4) whereas the samples prepared with cultures maintained in whey (Y_2 and Y_4) did not show any reduction in acidity when tested against control

Eventhough a slight difference was observed among these values statistical analysis showed that there was no significant difference in acidity between different samples (Table 18b)

The overall mean pH values for yogurt under different treatments are presented in Table 19a The mean values were found to be 4 20 ± 0 13 4 24 ± 0 15 4 26 ± 0 17 and 4 27 ± 0 11 respectively for samples under treatments Y_1 Y_2, Y_3 and Y_4

The lowest pH value of 4 20 was obtained for sample Y_1 and the highest value of 4 27 was obtained for Y_4 For Y_2 a value of 4 24 and for Y_3, the value of 4 26 was noted On statistical analysis the difference in the pH values were found to be not significant (Table 19b)

4 3 1 2 Tyrosine value

From the Table 20a it could be seen that the overall mean tyrosine values obtained for yogurt under treatments Y_1, Y_2, Y_3 and Y_4 were 0 40 ± 0 004 0 41 ± 0 01 0 37 ± 0 004 and 0 36 ± 0 01 mg/g respectively

Table 20a Tyrosine value of yogurt samples (mg/g) under different treatments

Replication	Treatment			
	Y_1	Y_2	Y_3	Y_4
1	0 38	0 36	0 39	0 38
2	0 46	0 46	0 38	0 36
3	0 39	0 44	0 36	0 40
4	0 38	0 39	0 36	0 36
5	0 39	0 41	0 36	0 32
6	0 38	0 39	0 38	0 36
Mean	0 40	0 41	0 37	0 36
S E ±	0 004	0 01	0 004	0 01

Table 20b Analysis of variance

Source	DF	SS	MS	F value
Between treatments	3	0 007	0 002	4 03 *
Within treatments	20	0 012	0 001	
Total	23	0 02		

* - Significant at 5 per cent level

A change in tyrosine value was noticed for yogurt under different treatments Yogurt under treatments Y_1 and Y_2 showed maximum proteolysis when compared to Y_3 and Y_4 Statistical analysis showed a significant difference between treatment and control yogurts Pairwise comparison between Y_1 and Y_2 revealed that there was no significant difference between them Same trend was noticed in the case of Y_3 and Y_4 also (Table 20b)

4 3 1 3 Total lactic count

The mean value of *S thermophilus* and *L delbrueckii* sub sp *bulgaricus* counts under different treatments are presented in Table 21a

From the table it could be seen that the mean total count of *S thermophilus* under four different treatments Y_1 Y_2, Y_3 and Y_4 were $2\ 42x10^6 \pm 0\ 91x10^5$, $3\ 59x10^5 \pm 2\ 12x10^5$, $1\ 91x10^8 \pm 0\ 62x10^5$ and $2\ 50x10^8 \pm 1\ 24x10^8$ cfu/ml respectively The corresponding figures for *L delbrueckii* sub sp *bulgaricus* were $2\ 38x10^8 \pm 0\ 63x10^6$ $1\ 75x10^8 \pm 0\ 67x10^6$ $2\ 25x10^6 \pm 1\ 04x10^5$ and $1\ 14x10^6 \pm 0\ 25x10^5$ cfu/g

The treatment products Y_1 and Y_2 prepared with lactose hydrolysed milk showed a better count of both *S thermophilus* and *L delbrueckii* sub sp *bulgaricus* when compared to control products (Y_3 and Y_4) However the maintenance of yogurt cultures in whey based media did not significantly

Table 21a Total count of yogurt samples (x10^6 cfu/ml) under different treatments

Replication	Treatment							
	Y_1		Y_2		Y_3		Y_4	
	S thermophilus	*L bulgaricus*	*S thermophilus*	*L bulgaricus*	*S thermophilus*	*L bulgaricus*	*S thermophilus*	*L bulgaricus*
1	5 60	4 50	2 56	1 72	0 92	0 87	0 93	0 89
2	2 20	2 40	0 15	0 12	1 72	2 80	3 20	1 34
3	1 92	0 15	1 28	1 22	0 41	0 41	0 38	0 30
4	4 50	3 70	14 0	4 20	4 80	7 20	8 40	2 14
5	1 82	1 70	0 75	0 67	1 86	1 40	1 11	1 16
6	0 22	1 80	2 80	2 60	1 72	0 86	0 97	0 98
Mean	2 42	2 38	3 59	1 75	1 91	2 25	2 50	1 14
S E +	0 91	0 63	2 12	0 67	0 62	1 04	1 24	0 25

Table 21 b1 Analysis of variance for *S thermophilus*

Source	DF	SS	MS	F-value
Between treatments	3	0 02563	0 00854	0 0286NS
Within treatments	20	5 9729	0 29864	

Table 21 b2 Analysis of variance for *L delbrueckii* sub sp *bulgaricus*

Source	DF	SS	MS	F-value
Between treatments	3	0 1945	0 0648	0 2054 NS
Within treatments	20	6 3128	0 3156	

NS - Not significant

affect their growth which could be confirmed by analysis of the data (Table 21 b1 and 21 b2)

4.3 1 4 Sensory evaluation

The mean organoleptic characteristics of yogurt under different treatments for general appearance, body and texture flavour and total scores are given in Table 22a

The mean score for general appearance under treatments Y_1, Y_2 Y_3 and Y_4 were 3 72 ± 0 18 4 10 ± 0 11, 3 91 ± 0 21 and 4 22 ± 0 13 respectively The general appearance score under treatments Y_2 and Y_4 were higher than Y_1 and Y_3

Although the general appearance of the yogurt samples prepared with the cultures maintained in whey was found to be slightly better than the samples prepared with cultures maintained in skim milk the difference between the treatments were found to be statistically not significant (Table 22 b1)

The body and texture score for yogurt under treatment Y_1 Y_2 Y_3 and Y_4 were 3 83 ± 0 14 4 15 ± 0 07 3 87 + 0 15 and 3 97 ± 0 13 respectively Here also the scores under treatments Y_2 and Y_4 were found to be better than treatments Y_1 and Y_3 (Table 22a) But the statistical analysis showed that the difference between treatments were not significant (Table 22 b2)

Table 22a Organoleptic scores obtained for yogurt samples under different treatments

Replication	Treatment Y_1				Y_2				Y_3				Y_4			
	A	B&T	F	TS	A	B&T	F	TS	A	B&T	F	TS	A	B&T	F	TS
1	3 00	3 33	5 32	11 66	4 33	4 00	8 00	16 33	3 66	3 66	6 00	13 33	4 33	4 33	8 66	17 33
2	4 00	4 00	7 00	14 75	4 50	4 25	8 50	17 25	3 50	3 50	7 50	14 50	3 75	3 50	9 00	15 25
3	3 75	4 00	8 50	16 25	3 75	4 00	9 00	16 75	4 00	3 75	8 00	15 75	4 25	4 00	7 50	15 75
4	4 30	3 66	8 00	16 00	4 00	4 33	8 00	16 33	4 66	4 33	8 66	17 66	4 66	4 00	8 00	16 66
5	3 66	4 33	8 66	16 66	4 00	4 33	8 66	17 00	4 33	4 33	8 66	17 33	4 30	4 30	8 66	17 33
6	3 66	3 66	6 66	14 00	4 00	4 00	6 00	14 00	3 33	3 66	6 00	13 33	4 00	3 66	6 00	13 66
Mean	3 72	3 83	7 34	14 88	4 10	4 15	8 02	16 28	3 91	3 87	7 47	15 32	4 22	3 97	7 98	15 99
S E +	0 18	0 14	0 26	0 76	0 11	0 07	0 22	0 48	0 21	0 15	0 25	0 78	0 13	0 13	0 22	0 58

A Appearance
B&T Body and texture
F flavour
TS Total score

Table 22 b1 Analysis of variance for general appearance score

Source	DF	SS	MS	F-value
Between treatments	3	0 832	0 277	1 784 NS
Within treatments	20	3 108	0 155	
Total	23	3 94		

Table 22 b2 Analysis of variance for body and texture score

Source	DF	SS	MS	F-value
Between treatments	3	0 368	0 128	1 241 NS
Within treatments	20	1 978	0 099	
Total	23	2 346		

NS - Not significant

Table 22 b3 Analysis of variance for flavour score

Source	DF	SS	MS	F-value
Between treatments	3	0 525	0 175	0 511 NS
Within treatments	20	6 853	0 343	
Total	23	7 379		

Table 22 b4 Analysis of variance for total score

Source	DF	SS	MS	F-value
Between treatments	3	7 217	2 406	0 913 NS
Within treatments	20	52 710	2 635	
Total	23	59 927		

NS - Not significant

The mean scores of yogurt samples for flavour was 7 34 ± 0 26 8 02 ± 0 22, 7 47 ± 0 25 and 7 98 ± 0 22 respectively for treatments Y_1 Y_2, Y_3 and Y_4 (Table 22a) For treatments Y_2 and Y_4 the score was found to be comparatively higher than treatments Y_1 and Y_3 On statistical analysis these differences were found to be not significant (Table 22 b3)

The overall scores obtained for yogurt samples under different treatments are given in Table 22a

Yogurt sample under treatment Y_1 got a mean overall score of 14 88 ± 0 76, for treatment Y_2 the mean score was 16 28 ± 0 48 For treatment Y_3 the score was 15 32 ± 0 78 and for treatment Y_4 a mean overall score of 15 99 ± 0 58 was obtained Although a higher mean overall score was observed for yogurt samples under treatments Y_2 and Y_4 the statistical analysis showed that there was no significant difference among yogurt samples under different treatments (Table 22 b4)

4 3 2 Bifidus yogurt

4 3 2 1 Acidity and pH

The overall mean values for acidity of bifidus yogurt under different treatments are presented in Table 23a From the table it could be seen that the overall mean values of acidity for bifidus yogurt under treatments B_1, B_2, B_3 and B_4

Table 23a Acidity of bifidus yogurt samples under different treatments (per cent of lactic acid)

Replication	Treatment			
	B_1	B_2	B_3	B_4
1	1 21	1 15	0 89	0 93
2	1 39	1 39	0 96	0 94
3	1 26	1 05	1 09	0 92
4	1 31	1 28	1 05	0 99
5	0 90	0 83	0 87	0 83
6	1 15	1 11	1 39	1 26
Mean	1 20	1 14	1 04	0 98
S E ±	0 07	0 08	0 08	0 06

Table 23b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 008	0 003	1 935 NS
Within treatments	20	0 029	0 001	
Total	23	0 037		

NS - Not significant

Table 24a pH of bifidus yogurt samples under different treatments

Replication	Treatment			
	B_1	B_2	B_3	B_4
1	4 10	4 21	4 55	4 51
2	4 02	4 10	4 38	4 64
3	4 18	4 21	4 26	4 58
4	4 06	4 11	4 18	4 39
5	4 58	4 64	4 45	4 46
6	4 34	4 21	4 02	4 08
Mean	4 21	4 25	4 31	4 44
S E ±	0 08	0 07	0 08	0 08

Table 24b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 186	0 062	1 533 NS
Within treatments	20	0 807	0 040	
Total	23	0 992		

NS - Not significant

were 1 20 ± 0 07 1 14 ± 0 08, 1 04 ± 0 08 and 0 98 ± 0 06 per cent of lactic acid respectively

The acidity developed by bifidus yogurt samples B_1 and B_2 were found to be higher when compared to B_3 and B_4 But when statistical analysis was carried out, the differences in values were found to be not significant (Table 23b)

The overall mean pH values for bifidus yogurt under different treatments are presented in Table 24a The mean values for pH were found to be 4 21 ± 0 08 4 25 ± 0 70 4 31 ± 0 08 and 4 44 ± 0 08 respectively for samples under treatments B_1 B_2, B_3 and B_4 However statistical analysis showed that there was no significant difference in pH values among different bifidus yogurt samples (Table 24b)

4 3 2 2 Tyrosine value

The overall mean tyrosine values for Bifidus yogurt under four different treatments are presented in Table 25a

From the Table it was observed that the values were 0 37 ± 0 03 0 35 ± 0 02 0 32 ± 0 03 and 0 32 ± 0 02 mg/g respectively for treatments B_1 B_2 B_3 and B_4 Proteolytic activity was found to be slightly better in treatment samples (B_1 and B_2) prepared with lactose hydrolysed milk in comparison to the control samples (B_3 and B_4) However, the difference

Table 25a Tyrosine value of bifidus yogurt samples under different treatments (mg/g)

Replication	Treatment			
	B_1	B_2	B_3	B_4
1	0 41	0 38	0 41	0 34
2	0 44	0 28	0 38	0 24
3	0 41	0 34	0 36	0 28
4	0 36	0 30	0 27	0 36
5	0 25	0 36	0 28	0 33
6	0 33	0 41	0 32	0 32
Mean	0 37	0 35	0 32	0 32
S E ±	0 03	0 02	0 03	0 02

Table 25b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 009	0 003	0 841 NS
Within treatments	20	0 075	0 004	
Total	23	0 085		

NS Not significant

in values were found to be statistically not significant (Table 25b)

4 3 2 3 Total lactic count

The mean lactic count of bifidus yogurt under different treatments are presented in Table 26a

From the Table it could be seen that the *S thermophilus* count for different treatments B_1 B_2 B_3 and B_4 were 2 57x10^8 ± 0 50x10^8, 2 56x10^8 ± 0 91x10^8 2 58x10^8 ± 0 39x10^8 and 2 25x10^8 ± 0 61x10^8 cfu/ml respectively The corresponding values for *L delbrueckii* sub sp *bulgaricus* were found to be 2 02x10^8 ± 0 41x10^8 2 03x10^8 ± 0 72x10^8 2 17x10^8 ± 0 46x10^8 and 1 97x10^8 ± 0 48x10^8 cfu/ml The rod coccus ratio for treatments B_1, B_2 B_3 and B_4 were 1 1 27 1 1 26 1 1 19 and 1 1 14 respectively A slightly better *S thermophilus* count was observed in all the four samples in comparison with *L delbrueckii* sub sp *bulgaricus* and *B bifidum B bifidum* count was found to be comparatively lower in all the four samples The values were 1 85x10^8 ± 0 44x10^8, 1 55x10^8 ± 0 46x10^8 1 44x10^8 ± 0 24x10^8 and 1 30x10^8 ± 0 26x10^8 cfu/ml respectively for treatments B B_2 B_3 and B_4 However no significant difference was noticed in total lactic count among the samples when statistical analysis was carried out (Table 26 b1 b2 and b3)

Table 26a Total count of Bifidus yogurt samples under different treatments (X10 cfu/ml)

Replication	Treatment											
	B			B_2			B			B		
	S thermo philus	*L bulgari cus*	*B bifidum*	*S thermo philus*	*L bulgari cus*	*B bifidum*	*S thermo philus*	*L bulgari cus*	*B bifidum*	*S thermo philus*	*L bulgari cus*	*B bifidum*
1	4 50	3 30	3 80	6 80	5 40	3 40	2 15	2 80	0 94	4 80	3 90	0 84
2	2 30	2 10	1 30	1 48	1 20	1 40	2 62	2 10	1 40	1 90	1 10	0 98
3	1 86	2 23	2 00	2 44	1 90	2 40	3 10	2 90	1 30	3 20	2 70	1 80
4	3 40	2 80	1 70	2 86	2 30	0 90	4 10	3 60	2 40	1 76	2 10	2 30
5	0 97	0 81	0 60	0 84	0 60	0 50	2 32	0 69	1 80	0 76	0 90	0 80
6	2 36	0 90	1 70	0 92	0 81	0 71	1 20	0 95	0 80	1 11	1 10	0 97
Mean	2 57	2 02	1 85	2 56	2 03	1 55	2 58	2 17	1 44	2 25	1 97	1 30
S E +	0 50	0 41	0 44	0 91	0 72	0 46	0 39	0 46	0 24	0 61	0 48	0 26

Table 26 b1 Analysis of variance for *S thermophilus*

Source	DF	SS	MS	F-value
Between treatments	3	0 0533	0 0177	0 2471NS
Within treatments	20	1 4387	0 0719	

Table 26 b2 Analysis of variance for *L delbrueckii* sub sp *bulgaricus*

Source	DF	SS	MS	F-value
Between treatments	3	1 0197	0 0065	0 0787NS
Within treatments	20	1 6735	0 0836	

Table 26 b3 Analysis of variance for *B bifidum*

Source	DF	SS	MS	F-value
Between treatments	3	0 0607	0 0202	0 3389NS
Within treatments	20	1 1958	0 0597	

NS - Not significant

4 3 2 4 Sensory evaluation

Table 27a shows the mean organoleptic characteristics of bifidus yogurt, under different treatments for general appearance, body and texture, flavour and total score

The mean scores for general appearance under treatments B_1 B_2, B_3 and B_4 were 3 93 ± 0 14 3 87 ± 0 19, 3 76 ± 0 20 and 3 88 ± 0 19 respectively The score for general appearance was found to be the lowest in sample B_3 and the values were almost same in other 3 samples On statistical analysis this difference was found to be not significant (Table 27 b1)

The overall mean scores for body and texture of bifidus yogurt under treatments B_1, B_2 B_3 and B_4 were 3 70 ± 0 12, 3 50 ± 0 07, 3 56 ± 0 25 and 3 45 ± 0 16 respectively Here also, the scores were found to be higher in B_1 and B_3 samples (Table 27a) When statistical analysis was carried out the differences in scores were found to be not significant (Table 27 b2)

The mean score for flavour was 7 66 ± 0 02, 7 22 ± 0 17, 7 34 ± 0 21 and 6 88 ± 0 16 respectively for bifidus yogurt samples under treatment B_1, B_2, B_3 and B_4 (Table 27a) For treatments B_1 and B_3 the score was found to be slightly higher than B_2 and B_4 On statistical analysis these differences were found to be not significant (Table 27 b3)

Table 27a Organoleptic scores obtained for bifidus yogurt samples under different treatments

Replication	Treatment															
	B				B_2				B_3				B_4			
	A	B&T	F	TS	A	B&T	F	TS	A	B&T	F	TS	A	B&T	F	TS
1	4 00	4 30	9 32	9 33	4 33	3 66	7 32	7 33	4 00	4 33	8 66	8 66	4 00	3 66	7 32	8 33
2	4 50	3 75	8 00	16 25	4 75	3 50	7 50	15 75	4 00	3 50	6 50	14 00	4 25	3 25	6 00	13 50
3	4 00	3 75	7 00	14 75	4 00	3 25	8 00	15 25	4 50	4 25	8 50	17 25	4 25	4 00	8 00	16 25
4	3 75	3 50	7 50	14 75	5 50	3 75	6 00	15 00	3 25	3 00	7 50	13 75	4 00	3 50	7 00	14 50
5	3 80	3 60	7 60	14 20	3 40	3 40	6 00	12 80	3 60	2 80	6 40	12 00	3 00	2 80	6 00	11 60
6	3 50	3 50	6 50	13 50	3 25	3 50	7 50	14 25	3 25	3 50	6 50	13 25	3 75	3 50	7 00	14 25
Mean	3 93	3 70	7 66	13 80	3 87	3 50	7 22	13 40	3 76	3 56	7 34	13 15	3 88	3 45	6 88	13 07
S E +	0 14	0 12	0 20	0 96	0 19	0 07	0 17	1 28	0 20	0 25	0 21	1 15	0 19	0 16	0 16	1 13

A Appearance
B&T Body and texture
F flavour
TS Total score

Table 27 b1 Analysis of variance for general appearance score

Source	DF	SS	MS	F-value
Between treatments	3	0 628	0 209	0 648 NS
Within treatments	20	6 463	0 323	
Total	23	7 091		

Table 27 b2 Analysis of variance for body and texture score

Source	DF	SS	MS	F-value
Between treatments	3	0 265	0 088	0 522 NS
Within treatments	20	3 390	0 169	
Total	23	3 655		

NS - Not significance

Table 27 b3 Analysis of variance for flavour score

Source	DF	SS	MS	F-value
Between treatments	3	0 521	0 171	0 817 NS
Within treatments	20	4 175	0 209	
Total	23	4 687		

Table 27 b4 Analysis of variance for total score

Source	DF	SS	MS	F-value
Between treatments	3	1 982	0 661	0 085 NS
Within treatments	20	155 177	7 759	
Total	23	157 159		

NS - Not significant

The mean overall scores for bifidus yogurt under treatments B_1, B_2 B_3 and B_4 were 13 8 ± 0 96 13 4 ± 1 28 13 15 ± 1 15 and 13 07 ± 1 13 respectively (Table 27a) Analysis of variance showed that there was no significant difference between these scores under different treatments (Table 27 b4)

4 3 3 Acidophilus milk

4 3 3 1 Acidity and pH

The overall mean acidity of acidophilus milk under different treatments are presented in Table 28a The mean values obtained for treatments A_1 A_2 A_3 and A_4 were 1 32 ± 0 08 1 34 ± 0 09 1 26 ± 0 09 and 1 24 ± 0 10 per cent of lactic acid respectively

Here a slight increase in acidity was noted in A_1 and A_2 (lactose hydrolysed products) when compared to A_3 and A_4 (unhydrolysed products) But statistical analysis revealed that there was no significant difference in acidity among the samples under different treatments (Table 28b)

The overall mean pH values for acidophilus milk under different treatments are given in Table 29a The mean values were found to be 3 98 ± 0 04 3 97 + 0 10 4 06 ± 0 03 and 4 09 ± 0 08 respectively for samples under treatment A_1 A_2, A_3 and A_4

Table 28a Acidity of acidophilus milk samples under different treatments (per cent of lactic acid)

Replication	Treatment			
	A_1	A_2	A_3	A_4
1	1 67	1 66	1 60	1 58
2	1 17	1 28	1 14	1 09
3	1 20	1 09	1 01	0 92
4	1 34	1 49	1 35	1 45
5	1 38	1 33	1 35	1 24
6	1 32	1 34	1 26	1 24
Mean	1 32	1 34	1 26	1 24
S E ±	0 08	0 09	0 09	0 10

Table 28b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 001	0 000	0 319 NS
Within treatments	20	0 026	0 001	
Total	23	0 028		

NS - Not significant

Table 29a pH of acidophilus milk samples under different treatments

Replication	Treatment			
	A_1	A_2	A_3	A_4
1	3 99	3 92	4 00	3 98
2	4 07	3 63	3 98	3 80
3	4 09	4 40	3 99	4 37
4	3 84	3 92	4 07	4 11
5	4 00	3 98	4 18	4 16
6	3 91	3 97	4 12	4 11
Mean	3 98	3 97	4 06	4 09
S E ±	0 04	0 10	0 03	0 08

Table 29b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 059	0 020	0 693 NS
Within treatments	20	0 564	0 028	
Total	23	0 623		

NS Not significant

Comparatively higher pH values were observed for samples prepared using whey based culture and the pH values were comparatively lower when culture was maintained in skim milk and lactose hydrolysed skim milk Eventhough slight difference in pH values were obtained in samples under different treatments, the statistical analysis revealed that there was no significant difference among them under different treatments (Table 29b)

4 3 3 2 Tyrosine value

The overall mean tyrosine value for acidophilus milk under different treatments are given in Table 30a The mean tyrosine values obtained for acidophilus milk under treatments A_1, A_2, A_3 and A_4 were 0 32 ± 0 02, 0 31 ± 0 02, 0 29 ± 0 02 and 0 27 ± 0 02 mg/g respectively

Acidophilus milk under treatments A_1 and A_2 caused maximum release of tyrosine, followed by A_3 and A_4 which indicated that the extend of proteolysis was comparatively higher in products prepared with lactose hydrolysed milk However, the values showed no significant difference when subjected to statistical analysis (Table 30b)

4 3 3 3 Total lactic count

The mean value for *L acidophilus* counts under different treatments are presented in Table 31a The counts under

Table 30a Tyrosine value of acidophilus milk samples under different treatments (mg/g)

Replication	Treatment			
	A_1	A_2	A_3	A_4
1	0 36	0 36	0 38	0 36
2	0 28	0 30	0 24	0 28
3	0 27	0 24	0 22	0 22
4	0 32	0 32	0 32	0 27
5	0 38	0 32	0 30	0 24
6	0 32	0 30	0 30	0 26
Mean	0 32	0 31	0 29	0 27
S E ±	0 02	0 02	0 02	0 02

Table 30b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 008	0 003	1 194 NS
Within treatments	20	0 045	0 002	
Total	23	0 053		

NS - Not significant

Table 31a Total count of acidophilus milk under different treatments ($x10^6$ cfu/ml)

Replication	Treatment			
	A_1	A_2	A_3	A_4
1	38 00	28 00	5 10	5 30
2	26 00	12 00	16 00	9 40
3	5 80	5 10	6 50	14 00
4	32 00	36 00	37 00	24 00
5	3 60	2 80	4 80	4 00
6	8 60	7 80	9 00	9 10
Mean	19 00	15 28	13 60	10 96
S E ±	6 04	5 52	5 07	5 52

Table 31b Analysis of variance

Source	DF	SS	MS	F-value
Between treatments	3	0 0911	0 0303	0 2152NS
Within treatments	20	2 8244	0 1412	

NS - Not significant

treatments A_1, A_2, A_3 and A_4 were 19 00x10^8 ± 6 04x10^8, 15 28x10^8 ± 5 52x10^8 13 60x10^8 ± 5 07x10^8 and 10 96x10^8 ± 5 52x10^8 cfu/ml respectively

A slight increase in *L acidophilus* count was observed in treatment samples (A_1 and A_2) when compared to control samples (A_3 and A_4) On statistical analysis these differences were found to be not significant (Table 31b)

4 3 3 4 Sensory evaluation

The average scores obtained for each parameter from five judges and from six replication under each treatment was subjected to statistical analysis

The mean overall scores for acidophilus milk for appearance, body and texture, and flavour was 4 40 ± 0 09, 4 40 ± 0 08 and 8 60 ± 0 16 respectively for sample A_1 4 17 ± 0 07, 4 25 ± 0 08 and 8 60 ± 0 12 for A_2 4 40 ± 0 11, 4 05 ± 0 12 and 8 00 ± 0 16 for A_3 and 4 35 ± 0 13, 4 05 ± 0 16 and 8 30 ± 0 19 for sample A_4 (Table 32a)

The figures in Table 32a indicated that there was a slight increase in scores for general appearance, body and texture and flavour in acidophilus milk samples under treatments A_1 and A_2 ie in treatment samples prepared with 50 per cent lactose hydrolysed milk when compared to A_3 and A_4

Table 32a Organoleptic scores obtained for Acidophilus milk samples under different treatments

Replication	Treatment															
	$\bar{A}$				A_2				$\bar{A}_3$				A			
	A	B&T	F	TS	A	B&T	F	TS	A	B&T	F	TS	A	B&T	F	TS
1	4 00	4 50	8 50	17 00	4 00	4 50	9 00	17 56	4 00	4 50	8 50	17 00	4 00	4 25	8 50	6 75
2	4 30	4 30	9 20	18 50	4 30	4 30	8 00	16 60	4 30	4 00	8 00	16 30	4 30	4 30	9 20	18 00
3	4 50	4 50	9 50	18 50	4 50	4 50	9 50	18 50	4 75	4 00	9 00	17 75	4 75	4 25	9 00	18 00
4	4 60	4 00	8 00	16 60	4 30	4 00	8 60	17 00	4 60	4 00	8 00	16 00	4 60	4 00	8 00	15 30
5	4 50	4 25	7 50	16 25	4 50	4 25	8 00	16 75	4 50	4 25	8 00	16 75	4 50	4 25	8 50	17 25
6	4 60	4 60	9 20	18 60	4 30	4 00	8 60	17 00	4 30	3 60	6 60	14 60	4 00	3 30	6 60	14 00
Mean	4 40	4 40	8 60	17 58	4 17	4 25	8 60	17 23	4 40	4 05	8 00	16 40	4 35	4 05	8 30	16 55
S E ±	0 09	0 08	0 16	0 44	0 07	0 08	0 12	0 29	0 11	0 12	0 16	0 44	0 13	0 16	0 19	0 65

A Appearance
B&T Body and texture
F flavour
TS Total score

Table 32 b1 Analysis of variance for general appearance score

Source	DF	SS	MS	F-value
Between treatments	3	0 039	0 013	0 204 NS
Within treatments	20	1 281	0 064	
Total	23	1 320		

Table 32 b2 Analysis of variance for body and texture score

Source	DF	SS	MS	F-value
Between treatments	3	0 405	0 135	1 594 NS
Within treatments	20	1 693	0 085	
Total	23	2 098		

NS - Not significant

Table 32 b3 Analysis of variance for flavour score

Source	DF	SS	MS	F-value
Between treatments	3	0 399	0 133	0 862 NS
Within treatments	20	3 088	0 154	
Total	23			

Table 32 b4 Analysis of variance for total score

Source	DF	SS	MS	F-value
Between treatments	3	5 569	1 856	1 389 NS
Within treatments	20	26 732	1 337	
Total	23	32 301		

NS - Not significant

Statistical analysis showed that these difference in scores were not significant (Table 32 b1 b2 and b3)

Acidophilus milk under treatment A_1 got a mean overall score of 17 58 ± 0 44 and for treatment A_2 the mean overall score was 17 23 ± 0 29 For treatment A_3 the value was 16 40 ± 0 44 and for treatment A_4 an overall score of 16 55 ± 0 65 was obtained (Table 32a) Statistical analysis showed that there was no significant difference in scores between samples under different treatments (Table 32 b4)

Discussion

DISCUSSION

5.1 Immobilized enzyme system

In recent years considerable attention is being directed in India as well as abroad towards the use of B-galactosidase in dairy industry, particularly in problems involving whey disposal, preparation of concentrated milk products, as well as in making low lactose milk/products for lactose intolerant populations of the world Usually B-galactosidases are used as soluble preparations and therefore cannot be reused and suffer from disadvantages like product inhibition, immunological reaction, low solubility and high cost Advantages associated with utilisation of immobilized enzyme include repeatability, better process control, adaptability to continuous use and good quality product free from enzyme Since whole cell lactases are more than ten fold less expensive than commercially available soluble lactase they have gained considerable importance during the past few years

Effective utilisation of whey, a by-product of cheese industry, is a serious problem for dairy industry Since enormous volumes of skim milk is being used for the preparation of bulk starter cultures, the possibilities of

utilisation of whey as a medium for culture maintenance is of considerable importance

Considering the above facts, an experiment was designed with the following objectives

1 Development of an immobilized β-galactosidase enzyme system using an efficient enzyme source and utilising simple techniques that are readily adaptable to dairy plants

2 Exploring the possibilities of utilisation of whey as a medium for culture maintenance

3 Preparation of lactose hydrolysed milk by using immobilized enzyme system and its utilisation for the preparation of fermented dairy products

5 1.1 β-galactosidase specific activity of different cultures

Three selected organisms viz *S thermophilus*, *L delbrueckii* sub sp *bulgaricus* and *K fragilis* were screened for their β-galactosidase specific activity and it was measured in terms of number of units The data pertaining to this parameter is given in Table 1

The mean β-galactosidase specific activity of permeabilized cells of *S thermophilus* was found to be 1 75 units The corresponding values for *L delbrueckii* sub sp *bulgaricus* and *K fragilis* were 1 25 and 2 46 units respectively, indicating a better activity of *K fragilis*

Savaiano and Levitt (1987) reported a β-galactosidase specific activity of 3 8 units for mixed yogurt cultures and the value for *S thermophilus* and *L delbrueckii* sub sp *bulgaricus* were 2 4 and 1 5 units/gram respectively

β-galactosidase specific activity observed for *S thermophilus* and *L delbrueckii* sub sp *bulgaricus* were almost agreeable with the reported value of Saviano and Levitt (1987) whereas in the case of *K fragilis* a low value of 2 43 units was obtained than the reported value of 3 11 units (Mahoney *et al* , 1975) They stated that 41 strains of *K fragilis* varied 60 fold in their ability to produce β-galactosidase This may be the possible reason for the reduction in β-galactosidase specific activity from the reported specific activity Since the specific activity of *K fragilis* was found to be comparatively better, it was selected for developing the immobilized enzyme system

5 1 2 Efficiency of the immobilized enzyme system

The data regarding the rate of lactose hydrolysis obtained with two types of immobilized enzyme system at fixed time intervals are shown in Table 2

From the table it could be seen that the rate of lactose hydrolysis is comparatively better with the immobilized system prepared using agar as immobilizing agent than the system prepared with sodium alginate A sudden reduction in lactose content was obtained within first half an hour using both the immobilized systems and thereafter the rate of hydrolysis slowly declined The efficiency at specific intervals of time was also found to vary Immobilized system prepared with agar hydrolysed 50 per cent lactose content present in 250 ml of milk within four hours whereas the system prepared with sodium alginate could not bring about 50 per cent lactose hydrolysis within four hours Only 45 per cent conversion was observed within the period of four hours

Rao *et al* (1988) reported that more than 95 per cent of sugar could be removed from a batch of 100 ml milk within 3 1/2 hours using an immobilized system prepared with 10 g *K fragilis* as source of enzyme and 2 per cent calcium alginate as immobilizing agent They also reported that the time required for sugar removal from milk could be

considerably reduced by changing the ratio of milk to immobilized yeast cells

The possible reason for a reduced conversion rate obtained in the present study may be due to a higher volume of milk used (250 ml)

From the results obtained it was found that the efficiency of the system prepared with agar as immobilizing agent was better than the other system So it was selected for further studies

5 1 3 Repeatability of the system

The system was used repeatedly in five batches for hydrolysis of lactose in milk without change in its activity or mechanical stability of the beads However, a gradual reduction in activity was observed after fifth passing A slight brownish discolouration was also noticed on the beads when the column was stored for long period

The long period of storage of the column during the trials may be the possible reason for the reduction in activity of the system

5.2 Performance of starter cultures in different media

5.2 1 Change in acidity and pH

5.2.1.1 S. thermophilus

The overall mean acidity developed by *S thermophilus* culture in four different media, M_1, M_2, M_3 and M_4 is presented in Table 3a

From the table it could be seen that, *S thermophilus* developed a comparatively higher acidity when grown in whey based media (M_3 and M_4), compared to skim milk (M_1 and M_2) This observation is in agreement with the findings of Mathur and Shahani (1979) They reported that whey based media with seven per cent total solids supported better growth of *S thermophilus* and certain other lactic acid bacteria, than media prepared with reconstituted skim milk with 10 per cent total solids or ordinary milk

In between M_3 and M_4 a better development of acidity was observed in M_4 (lactose hydrolysed whey) This observation is in line with the report of Marschke and Dulley (1978) They observed a faster acid development of lactic acid bacteria in lactose hydrolysed milk than control The probable reason for this finding may be the stimulatory action of some of the limiting amino acids that are available in condensed whey

Broome et *al* (1982) also have observed that condensed whey could stimulate the activity of *S thermophilus*

The results obtained can be further confirmed by noting the pH values in different media The mean pH values were found to be 4 41 ± 0 07 4 35 ± 0 04 4 33 ± 0 08 and 4 31 ± 0 09 respectively when *S thermophilus* was grown in media M_1 M_2 M_3 and M_4 (Table 8a) Eventhough no significant difference was noticed between treatments a comparatively lower pH value was observed for *S thermophilus* in media M_3 and M_4 indicating faster acid development and shorter curd setting time

5 2 1 2 *L delbrueckii* sub sp *bulgaricus*

The mean lactic acid percentage produced by *L delbrueckii* sub sp *bulgaricus* in different media are presented in Table 4a

The overall mean acidity developed by *L delbrueckii* sub sp *bulgaricus* was found to be comparatively better when grown in skim milk (M_1), especially in lactose hydrolysed skim milk (M_2) than in whey This result did not confirm the claim of Mathur and Shahani (1979) They observed a better growth of *L delbrueckii* sub sp *bulgaricus* in whey containing seven per cent total solids

Reddy *et al* (1976) reported that the use of different strains of *L delbrueckii* sub sp *bulgaricus* has resulted in the production of different levels of acidity in whey The possible reason for a decline in acidity in whey media in the present study may be due to variation in the strain However no significant differences were noticed among the four medias indicating the replacement of skim milk with whey/lactose hydrolysed whey as a culture media for *L delbrueckii* sub sp *bulgaricus*

The results obtained regarding acidity can be further compared with the pH values obtained under different treatments (Table 9a) The mean pH values were found to be 3 52 ± 0 03, 3 52 ± 0 04, 3 60 ± 0 02 and 3 55 ± 0 04 respectively when grown in media M_1 M_2 M_3 and M_4

5 2 1 3 *B bifidum*

The overall mean acidity developed by *B bifidum* is shown in Table 5a From the table it could be observed that, the acidity produced by *B bifidum* can be enhanced by growing in media M_3 and M_4 (whey and lactose hydrolysed whey) This result is in accordance with the observation of Cheng and Sandine (1989) and Gorre *et al* (1992)

Cheng and Sandine (1989) reported that a whey based medium with seven per cent sweet whey 0 05 per cent cysteine

and 0 3 per cent yeast extract would be satisfactory for the growth of a variety of bifidobacterium species

Gorre *et al* (1992) in their attempt to produce a low cost medium for production of *B bifidum* found out that a 15 fold improvement on batch productivity could be achieved using a whey based medium

The results obtained in the present study can be further confirmed by observing the pH values under different treatments The mean pH values for *B bifidum* when grown in media M_1, M_2, M_3 and M_4 were 3 61 $\pm$ 0 06 3 51 $\pm$ 0 07, 3 46 $\pm$ 0 05 and 3 42 $\pm$ 0 03 respectively Magdalenic and Krdev (1990) suggested that addition of whey retentate to milk retentate could result in better growth of *B bifidum* and reduced fermentation time This result also support the findings in the present study

The possible reason attributed for enhanced performance of this culture in whey media may be due to the release of bifidus growth stimulating factors present in rennet whey The results obtained in the present study regarding the performance of *B bifidum* in whey indicates that whey can be suggested as a better medium for maintenance of this culture

5 2.1 4 *L. lactis*

The overall mean acidity developed by *L lactis* in different media M_1, M_2, M_3 and M_4 are presented in Table 6a

A better development of acidity was observed, when the culture was maintained in whey based media This finding is agreeable with the observation of Christopherson and Zottola (1989) They observed that *L lactis* C_2 strain could be propagated successfully in whey permeate with added yeast extract at 30°C A final pH of 4 67 and cell numbers of 9 5 $\log_{10}$ cells/ ml was achieved when grown in this media

The overall mean pH values obtained in the present study when *L lactis* was grown in media M_1, M_2 M_3 and M_4 were 4 35 ± 0 08, 4 25 ± 0 06, 4 20 ± 0 05 and 4 18 ± 0 05 respectively (Table 11a) These values also support the observation regarding acidity and total count

The possible reason for enhanced acidity and growth rate when whey was selected as growth medium may be due to the availability of some of the limiting aminoacids in condensed whey

5 2 1 5 *L acidophilus*

The overall mean acidity developed by *L acidophilus* in different media is presented in Table 7a The data pertaining the pH values are given in Table 12a

From the tables it could be seen that the performance of *L acidophilus* was better in skim milk when compared to whey However no significant differences were noted among the media indicating that skim milk can be successfully replaced by whey as a media for maintenance of *L acidophilus*

5 2 2 **Total count**

5 2 2 1 *S thermophilus*

The mean total count of *S thermophilus* when maintained in media M_1 M_2 M_3 and M were 4 11x10^8 ± 0 42x10^8 4 18x10^8 ± 0 41x10^8 4 24x10^8 ± 0 57x10^8 and 4 34x10^8 + 0 64x10^8 cfu/ml respectively

It could be seen from the table that whey media stimulated the growth of *S thermophilus* when compared to skim milk This result is in accordance with the observation of Broome *et al* (1982) They recommended whey protein as a growth stimulant of *S thermophilus* TS_2 and *Lactobacillus helveticus* LB Some of the limiting amino acids available in condensed cheese whey might have contributed to the better

growth of *S thermophilus* and certain other lactic acid bacteria

5 2 2 2 *L delbrueckii* sub sp *bulgaricus*

The data pertaining to *L delbrueckii* sub sp *bulgaricus* count when grown in different media are presented in Table 14a

A slightly enhanced activity of *L delbrueckii* sub sp *bulgaricus* was noticed in skim milk especially in lactose hydrolysed milk, when compared to whey (M_3) or lactose hydrolysed whey (M_4) media However no significant difference was noticed among the four different media indicating that skim milk could be successfully replaced by whey as a medium for maintenance of *L bulgaricus*

5 2 2.3 *B bifidum*

The overall mean *B bifidum* count when grown in media M_1, M_2, M_3 and M_4 are presented in Table 15a The values obtained were $4\ 14x10^8 \pm 0\ 90x10^8$, $4\ 26x10^8 \pm 0\ 42x10^8$, $4\ 67x10^8 \pm 0\ 35x10^8$ and $4\ 84x10^8 \pm 0\ 44x10^8$ respectively when grown in media M_1 M_2, M_3 and M_4

In media M_3 and M_4 (whey based media) a better activity of bifidobacteria was observed when compared to media M and M_2 This observation is agreeable with the report of Cheng

and Sandine (1989), Gorre *et al* (1992) and Baig and Prasad (1995) According to Baig and Prasad (1995) whey solids in yoghurt were stimulatory to the growth of *S thermophilus* and *B bifidum*

Misra and Kuila (1991) observed a viable count of 4x10^9 cfu/ml for *B bifidum* after inoculating with 10 per cent level of inoculum and incubating at 37 C for 18 hours, whereas in the present study a slightly lower count was observed in all the four media This could be due to low inoculum percentage used in the study (two per cent) However, Anita *et al* (1987) suggested that rennet whey contained some growth stimulatory factors such as glycopeptides which might be the possible reason for the better performance of this organism in media M_3 and M_4

5 2 2 4 *L lactis*

The data pertaining to *L lactis* count in different media are presented in Table 16a The mean total counts of *L lactis* under different treatments M_1, M_2 M_3 and M_4 were 5 50x10^8 ± 0 75x10^8, 5 55x10^8 ± 0 60x10^8, 5 50x10^8 ± 0 59x10^8 and 5 58x10^8 ± 0 67x10^8 respectively

Christopherson and Zottola (1989) observed a higher viable count of *L lactis* C_6 strain when grown in whey at their optimum time temperature combination whereas in the

present study, the growth rate of the culture was found to be the same in all the four media indicating that *L lactis* could be successfully maintained in whey without affecting its activity or growth rate

5.2.2 5 *L. acidophilus*

The overall mean total count of *L acidophilus* were 54 05x10^8 ± 4 15x10^8, 55 78x10^8 ± 4 79x10^8, 10 96x10^8 ± 2 71x10^8 and 12 99x10^8 ± 3 56x10^8 cfu/ml respectively when grown in media M_1 M_2, M_3 and M_4 A significantly higher total count was observed in media M_1 and M_2

Mathur and Shahani (1979) observed a mean *L acidophilus* count of 9 81x10^8 cfu/ml in whey media with seven per cent total solids and 2 90x10^8 cfu/ml in skim milk with ten per cent total solids Eventhough the growth rate of *L acodophilus* was comparatively less in whey based media when compared to skim milk media the culture could produce a reasonable number of organisms in whey based media also

In the present study when five selected starter cultures were tested for their performance in four different media it was found that whey and lactose hydrolysed whey slightly enhanced the activity of cultures, especially *S thermophilus L lactis* and *B bifidum* Eventhough *L delbrueckii* sub sp *bulgaricus* and *L acidophilus* performed better in lactose

hydrolysed skim milk no significant difference was noted among the four media indicating that skim milk could be completely replaced by whey/lactose hydrolysed whey for their maintenance

5.3 Biochemical and microbiological qualities of low lactose fermented milk products

5 3 1 Yogurt

5 3 1 1 Acidity and pH

S thermophilus and *L delbrueckii* sub sp *bulgaricus* are homofermentative organisms In the mixed culture they are capable of producing optimum range of lactic acid under favourable conditions of incubation i e at 30 C for long set or 42 C for short set method

The overall mean acidity of different yoghurt samples prepared by different techniques are presented in Table 18a

From the table it could be seen that the mean titratable acidity of yoghurt samples under different treatments were 1 13 + 0 05 1 10 ± 0 04 1 08 ± 0 09 and 1 0 ± 0 04 per cent of lactic acid respectively for treatments Y_1, Y_2 Y_3 and Y_4

Baig (1994) observed an acidity of 0 99 + 0 06 per cent of lactic acid for control yoghurt at a pH of 4 6 Tamine and

Robinson (1985) have also recommended mild yoghurts of pH 4 4 to 4 6 and an acidity of 0 9 per cent lactic acid for consumption

In the present study the control yoghurt samples (Y_3 and Y_4) prepared with unhydrolysed milk showed a slightly higher acidity than the values suggested by Tamime and Robinson (1980) The enhanced activity may be due to the long incubation period adopted Tamime and Robinson incubated their cultures at 42 C for 4 1/2 hours whereas in the present study the temperature time combination adopted was 30 C for 16 hours

The treatment yoghurt samples (Y_1 and Y_2) prepared using 50 per cent lactose hydrolysed milk showed a slightly enhanced activity when compared to samples Y_3 and Y_4 The possible reason for enhanced acidity might be the elimination of steps in substrate catabolism for yoghurt cultures and faster growth of bacterial cultures resulting in more acid production

The result obtained for titratable acidity could be further confirmed by noting the pH values of the samples under different treatments The mean pH values were found to be 4 20 $\pm$ 0 13 4 24 $\pm$ 0 15 4 26 $\pm$ 0 17 and 4 27 $\pm$ 0 11 respectively for treatments Y_1 Y_2 Y_3 and Y_4 (Table 19a) Eventhough slightly higher pH values were noted for samples

under treatment Y_3 and Y_4, statistically these differences were found to be not significant

5 3 1 2 Tyrosine value

The mean tyrosine value of yoghurt samples under different treatments are presented in Table 20a The amount of tyrosine liberated by yoghurt samples (Y_3 and Y_4) were in agreement with the values observed by Rajagopal and Sandine (1990) They reported that *L delbrueckii* sub sp *bulgaricus* were highly proteolytic than *S thermophilus* and that mixed cultures of *S thermophilus* and *L delbrueckii* sub sp *bulgaricus* liberated more tyrosine (92 to 419 9 ug/ml) than the sum of individual cultures

Tamime and Deeth (1980) stated that the proteolysis in yoghurt was dependant upon the strain of starter culture and the ratio between *S thermophilus* and *L bulgaricus*

The treatment samples (Y_1 and Y_2) prepared with 50 per cent lactose hydrolysed milk showed a significantly higher proteolytic activity than control samples (Y_3 and Y_4) This finding is in co-relation with the report of Grooda *et al* (1983) and Marchke *et al* (1980) They observed an increase in proteolysis in the enzyme treated cheddar cheese

The enhanced proteolytic activity of yoghurt cultures in lactose hydrolysed milk may be due to the faster growth of the organisms, utilizing the simplest form of sugars available in hydrolysed milk

5 3 1 3 Total lactic count

The mean total lactic count of yoghurt samples under different treatments are presented in Table 21a

From the table it could be seen that the mean total count of *S thermophilus* under treatments Y_1 Y_2, Y_3 and Y_4 were $2\ 42x10^8 \pm 0\ 91x10^8$, $3\ 59x10^8 \pm 2\ 12x10^8$ $1\ 91x10^8 \pm 0\ 62x10^8$ and $2\ 50x10^8 \pm 1\ 24x10^8$ cfu/ml respectively The corresponding figures for *L delbrueckii* sub sp *bulgaricus* were $2\ 38x10^8 \pm 0\ 63x10^8$ $1\ 75x10^8 \pm 0\ 67x10^8$ $2\ 25x10^8 + 1\ 04x10^8$ and $1\ 14x10^8 \pm 0\ 25x10^8$ cfu/ml The values indicated that, the starter cultures used in the preparation of yoghurt were very active and produced a reasonable number of organisms when the acidity was around one per cent lactic acid

Tamime and Robinson (1985) suggested that yoghurt should contain more than $100x10^6$ organisms/ml at the time of consumption of the product

In the present study an optimum growth of *S thermophilus* and *L delbrueckii* sub sp *bulgaricus* were obtained under all

the four treatments The treatment products prepared with lactose hydrolysed milk showed a better growth of *S thermophilus* and *L delbrueckii* sub sp *bulgaricus* However, a contradictory report was given by Abd-El-Hady *et al* (1985) that lactose hydrolysis had an inhibitory effect on growth of mixed cultures

5 3 1 4 Sensory evaluation

The organoleptic characteristics of yoghurt under different treatments were evaluated in terms of scores obtained for general appearance body and texture and flavour by a panel of five judges The scheme proposed by Pearce and heap (1974) was followed for the evaluation

The mean scores obtained for general appearance body and texture and flavour are presented in Table 22a Eventhough the score for general appearance for yoghurt under treatments Y_2 and Y_4 were higher statistically this difference was found to be not significant A similar trend was observed for body and texture also The body and texture score may indicate the quality of yoghurt in terms of viscosity curd tension gel firmness and syneresis The present study indicated that body and texture of yoghuit was not affected by lactose hydrolysis of milk or by using cultures maintained in whey based media With regard to flavour score the values under treatments Y_2 and Y_4 were found to be higher However no significant

difference was noticed among the samples indicating that yoghurt under all the treatments produced a satisfactory level of flavour compounds Similarly it could be assumed that the repeated use of immobilized column had not resulted in development of any type of off flavours

The mean overall score for yoghurt under treatment Y , Y_2, Y_3 and Y_4 were 14 88 ± 0 76 16 28 ± 0 48 15 32 ± 0 78 and 15 99 ± 0 58 respectively Higher total scores under treatment Y_2 and Y_4 were due to higher score for general appearance body and texture and flavour

From these results obtained the following conclusion can be made that yoghurt prepared using lactose hydrolysed milk with cultures maintained in lactose hydrolysed whey was ideal and was equally acceptable with control product prepared using ordinary milk and cultures maintained in skim milk The former product had an added advantage that it contains less quantity of lactose

5 3 2 Bifidus yoghurt

5 3 2 1 Acidity and pH

Bifidobacteria were reported to produce lactic acid and acetic acid in 2 3 ratio (Scardori 1986) So the values of titratable acidity obtained here include both lactic acid and acetic acid

The mean titratable acidity of bifidus yoghurt under different treatments B_1, B_2 B_3 and B_4 were 1 20 ± 0 07 1 14 ± 0 08, 1 04 ± 0 08 and 0 98 ± 0 06 per cent of lactic acid respectively Baig (1994) observed an acidity of 0 98 + 0 04 for bifidus yoghurt at a pH of 4 6 The acidity obtained for control products (B_3 and B_4) in the present study was in accordance with the reported figures of Baig (1994) However a slight increase in acidity was observed in treatment samples B_1 and B_2 prepared with lactose hydrolysed milk, when compared to B_3 and B_4 This result was agreeable with the observation of O'Leary and Woychik (1976) They reported that lactose hydrolysed yoghurt had more lactic acid than control prepared with unhydrolysed milk The possible reason for enhanced performance of the cultures in lactose hydrolysed milk might be the faster growth of cultures in the presence of easily available carbohydrates

A similar trend as observed in acidity was noted in pH also The mean pH of bifidus yoghurt under different treatments B_1, B_2 B_3 and B_4 are presented in Table 24a

A slightly lower pH values were observed in treatment samples (B and B_2) in comparison with B_3 and B_4 This result is comparable with the observation of Gyuriesek and Thompson (1976) They stated that as the per cent of lactose hydrolysis increased the time of curd setting decreased by

about 40 minutes Abdou-Sonia *et al* (1984) has also observed that the use of lactose hydrolysed milk in the preparation of a particular variety of cheese decreased coagulation time considerably The possible explanation for the shorter time to attain a specified pH for hydrolysed milk may include the availability of easily metabolisable nutrients, favouring better growth of starter cultures and faster acid development

5.3.2.2 Tyrosine value

Proteolysis in bifidus yoghurt under different treatments in the present study was measured in terms of tyrosine value and are presented in Table 20a

From the table it could be seen that the overall mean tyrosine value of bifidus yoghurt under treatments B_3 and B_4 were in agreement with the values reported by Misra and Kuila (1991) According to them, tyrosine value of milk fermented by *B bifidum* isolated from the infant faecal sample was in the range of 202 to 286 ug/g after 24 hours of incubation The values obtained with the strains from NDRI, Karnal and National collection of Dairy organisms, Reading, were 245 to 371 5 ug/g

The treatment samples (B_1 and B_2) prepared with 50 per cent lactose hydrolysed milk showed a slightly higher proteolytic activity then control (B_3 and B_4) This finding

is in line with the observation of Marschke *et al* (1980) They reported that addition of commercial B-galactosidase preparation to cheese milk could result in increased proteolytic activity

The proteolytic activity of different bifidus yoghurt samples were comparatively lower than corresponding values of yoghurt samples This may be due to the weak proteolytic activity of bifidobacteria as reported by Kurmann (1988) or due to an imbalance in ratio between *S thermophilus* and *L delbrueckii* sub sp *bulgaricus*

5 3 2 3 Total lactic count

The mean total count of *S thermophilus L delbrueckii* sub sp *bulgaricus* and *B bifidum* are given in Table 26a

From the table it could be seen that, the count of *S thermophilus* was comparatively higher than *L delbrueckii* sub sp *bulgaricus* and *B bifidum* Growth and activity of *S thermophilus* was found to be higher in B_1 B_2, B_3 and B_4 in comparison with Y_1 Y_2 Y_3 and Y_4 This finding is in agreement with the observation of Murti *et al* (1992) and Baig (1994) They found that *S thermophilus* developed better in the presence of bifidobacteria while *L delbrueckii* sub sp *bulgaricus* growth was found to get inhibited

The rod cocci ratio for treatments B_1, B_2, B_3 and B_4 were 1 1 27, 1 1 26, 1 1 19 and 1 1 4 respectively When treatment and control samples were compared, no significant difference was observed in total lactic count among the four different samples

5 3 2 4 Sensory evaluation

The sensory evaluation of bifidus yoghurt was carried out on the basis of score for general appearance body and texture and flavour

The treatment products B_1 and B_2 scored higher for the parameters general appearance, body and texture as well as for flavour resulting in higher total score (13 8 $\pm$ 0 96 for B_1 and 13 4 $\pm$ 1 28 for B_2) The total score remained comparatively lower for control products B_3 and B_4

These results are in agreement with the observation of Woodward and Kosikowski (1975) who reported that the body and texture and flavour scores were better for cheese prepared with lactose hydrolysed milk, than those prepared with unhydrolysed milk O'Leary and Woychik (1976) have also reported that a higher flavour score was obtained in cheese prepared with lactose hydrolysed milk

From the foregoing discussion it can be concluded that, good quality Bifidus yoguhrt with satisfactory organoleptic characteristics could be prepared using lactose hydrolysed milk No undesirable effect was noticed in the products prepared with cultures maintained in whey/lactose hydrolysed whey showing promising results in bifidus yoghurt production

5 3 3 Acidophilus milk

5 3 3 1 Acidity and pH

The mean titrable acidity of Acidophilus milk for treatment A_1, A_2 A_3 and A_4 were 1 32 ± 0 08 1 34 ± 0 09, 1 26 ± 0 09 and 1 24 ± 0 10 per cent of lactic acid respectively This result is comparable with the observations made by Rao and Gandhi (1987)

A slight higher acidity was observed in treatment samples (A_1 and A_2) prepared with 50 per cent lactose hydrolysed milk This was in concurrence with the observations made by Oh *et al* (1991) who reported that during fermentation with single culture B-galactosidase treatment of milk slightly stimulated viable cell numbers and acid production of *L acidophilus*

This was further confirmed by noting the pH values of the samples under different treatments The mean pH values were 3 98 ± 0 04 3 97 ± 0 10 4 06 ± 0 03 and 4 09 ± 0 08 respectively for treatments A_1 A_2 A_3 and A_4 This value is

agreeable with observations of Gandhi and Rao (1989) They reported a standard pH of 3 9 to 4 0 for acidophilus milk A slightly lower value of pH was observed for treatment samples (A_1 and A_2) This decline in pH may be due to a higher acid production as a result of faster growth of bacterial culture

5 3 3 2 Tyrosine value

Proteolytic activity of acidophilus milk under different treatments are presented in Table 30a

From the table it was observed that the tyrosine value of acidophilus milk under treatments A_1 A_2, A_3 and A_4 were 0 32 ± 0 02 0 31 ± 0 02, 0 29 ± 0 02 and 0 27 ± 0 02 mg/g respectively This result is in line with the observation of Geetha and Khan (1994) They reported a tyrosine value ranging from 0 224 to 0 381 mg/g for acidophilus milk

Acidophilus milk under treatments A and A_2 caused maximum release of tyrosine followed by A_3 and A_4 which indicated that the extent of proteolysis was comparatively higher in treatment products when compared to control This finding is in correlation with the observation of Marchke *et al* (1980) They reported that addition of B-galactosidase to cheese milk resulted in increased proteolytic break down in cheese

5 3 3 3 Total count

The mean total count of *L acidophilus* under different treatments are presented in Table 31a

The mean acidophilus count was found to be 19 00x10^8 ± 6 04x10^8, 15 28x10^8 ± 5 52x10^8 13 60x10^8 ± 5 07x10^8 and 10 96x10^8 ± 5 52x10^8 cfu/g respectively for treatments A_1 A_2 A_3 and A_4 Better count of *L acidophilus* observed in treatment samples (A_1 and A_2) is in accordance with the report of Oh *et al* (1991) They suggested that during fermentation with single cultures of *L acidophilus* B-galactosidase treatment of milk slightly stimulated viable cell numbers

Another favourable result observed was that the products prepared with cultures maintained in whey did not show any significant decline in activity of *L acidophilus*

5 3 3 4 Sensory evaluation

The organoleptic characteristics of acidophilus milk under different treatments were evaluated in terms of scores obtained for general appearance, body and texture and flavour by a panel of five judges

The mean values obtained for total score under four different treatments A_1 A_2 A_3 and A_4 were 17 58 ± 0 44 17 23 ± 0 29 16 40 ± 0 44 and 16 55 ± 0 65 respectively The

treatment products (A_1 and A_2) scored better when compared to control (A_3 and A_4) A similar trend was observed for the scores obtained for body and texture as well as for flavour A low total score for control products when compared to treatment products could be explained by the fact that control samples scored less values for body and texture and flavour

From the results obtained it could be considered that acidophilus milk prepared with lactose hydrolysed milk and cultures maintained in lactose hydrolysed whey was ideal and was equally acceptable with ordinary acidophilus milk, prepared with unhydrolysed lactose and cultures maintained in skim milk

From the foregoing discussion, it could therefore be concluded that

1 Permeabilized cells of *K fragilis* possessed better B-galactosidase specific activity than the cells of *S thermophilus and* L *delbrueckii* sub sp *bulgaricus*

2 An efficient immobilised B-galactosidase enzyme system could be developed using *K fragilis* cells, and food grade agar as immobilizing agent and this inturn was utilised for hydrolysing lactose content in milk This system could be repeatedly used in five batches for the

...tion in dairy industry therefore has a bright future

hydrolysis of lactose in milk without change in efficiency or in mechanical stability of the beads

3 All the five selected standard cultures of lactic acid bacteria showed a better performance in whey based media, suggesting replacement of skim milk completely with whey/lactose hydrolysed whey as a medium for culture maintenance

4 Good quality fermented milk products like yoghurt bifidus yoghurt and acidophilus milk with low lactose could be prepared using the milk obtained by passing through immobilized cell column No organoleptic changes were observed in these products when compared with controls No technological problems were also observed Acidity, proteolytic activity and growth rate were found to be slightly enhanced by lactose hydrolysis

Future prospects

Although the field of enzyme immobilization appears to be the most attractive from the point of view of their regenerative nature and low cost the large scale use of this technology in dairy industry has not been sufficiently explored in India A venture into the field of enzyme immobilization in India, particularly from the point of its application in dairy industry therefore has a bright future

The immobilization technology has the advantage of reducing the requirement of enzyme in a biochemical process because of the possibilities of reuse However difficulties are encountered in preserving the activities of immobilized enzymes over longer periods of time due to their proteinaceous nature Further research is needed in this aspect

There are many reports in all parts of the world regarding the utilization of whey as a medium for propagating starter cultures particularly for the development of phage inhibitory media But in India this technique is relatively under developed and even reports are scanty in this aspect To expand the scope of utilisation of whey as a culture medium additional investigations are needed However we can expect the successful adoption of this new technology in the near future

M_1 - Media prepared using ordinary skim milk

M_2 - Media prepared using 50 per cent lactose hydrolysed skim milk

M_3 - Media prepared using condensed whey with 12 per cent total solids

M_4 - Media prepared using 50 per cent lactose hydrolysed condensed whey

A_1, B_1, Y_1 - Products prepared with 50 per cent lactose hydrolysed milk and cultures maintained in 50 per cent lactose hydrolysed skim milk

A_2, B_2, Y_2 - Products prepared with 50 per cent lactose hydrolysed milk and cultures maintained in 50 per cent lactose hydrolysed whey

A_3, B_3, Y_3 - Products prepared with ordinary milk and cultures maintained in 50 per cent lactose hydrolysed skim milk

A_4, B_4, Y_4 - Products prepared with ordinary milk and cultures maintained in 50 per cent lactose hydrolysed whey

Summary

SUMMARY

Three selected organisms viz *S thermophilus* *L delbrueckii* sub sp *bulgaricus* and *K fragilis* were screened for their B-galactosidase specific activity and *K fragilis* was selected as the best from among them Suitability of sodium alginate as well as agar as immobilizing agents were also screened An immobilized B-galactosidase enzyme system was prepared using permeabilized cells of *K fragilis* as an enzyme source and food grade agar as an immobilizing agent This was utilised for hydrolysing lactose present in milk or whey which in turn was used for the preparation of fermented milk products with low lactose An attempt was also made to explore the possibility of utilisation of whey as a medium for culture maintenance

On screening three selected organisms for their B-galactosidase specific activity the activity was found to be the highest for *K fragilis* Similarly food grade agar was found to be a safe economical and efficient immobilizing agent On estimating the rate of lactose hydrolysis a sudden decline in lactose content was observed within first 30 minutes but there after the rate of lactose hydrolysis was very slow The immobilized enzyme system prepared with 10 g of *K fragilis* cells hydrolysed 50 per cent of lactose content present in 250 ml of milk after holding in the column for

four hours at 30 C This system was used repeatedly in five batches for the hydrolysis of lactose without any decline in activity However a reduction in activity was observed after fifth passing A slight brownish discolouration was also noticed on the beads

On screening five selected cultures of lactic acid bacteria for their performance in whey based media it was found that *S thermophilus*, *L delbrueckii* sub sp *bulgaricus* *B bifidum*, *L lactis* and *L acidophilus* varied in their ability to grow in whey based medium Whey alone was a poor medium but addition of yeast extract and cysteine improved the growth and activity of starter cultures *S thermophilus* *L lactis* and *B bifidum* showed a slightly enhanced activity in whey based media especially in 50 per cent lactose hydrolysed whey, whereas *L delbrueckii* sub sp *bulgaricus* and *L acidophilus* performed better in lactose hydrolysed skim milk However, no significant difference was noted among the four media indicating that skim milk can be successfully replaced by whey especially lactose hydrolysed whey

Three different products viz yogurt bifidus yogurt and acidophilus milk were prepared utilising 50 per cent lactose hydrolysed milk and ordinary milk Cultures maintained separately in lactose hydrolysed skim milk (M_2) and lactose hydrolysed whey (M_4) were used for product preparation

because the performance of the cultures were found to be better in M_2 and M_4 among the four media screened All the three products under different treatment (Y_1, Y_2, Y_3, Y_4 B_1 B_2 B_3 B_4 and A_1 A_2, A_3, A_4) were analysed for acidity pH tyrosine value and total count The products were also subjected to organoleptic evaluation

The utilisation of lactose hydrolysed milk for product preparation slightly enhanced acidity proteolytic activity and viable count in all the three products

A significantly higher proteolytic activity was observed in treatment yogurts Y_1 and Y_2 prepared with lactose hydrolysed milk

Organoleptic evaluation of yogurt under treatments, Y_2 and Y_4 got a slightly higher total score than Y_1 and Y_3 prooving that lactose hydrolysed milk and cultures maintained in lactose hydrolysed whey can be successfully used for production of fermented milks

Appearance body and texture, and flavour of bifidus yogurt as well as acidophilus milk under all different treatments when compared, it was found that there was no significant difference among them indicating that they were equally acceptable

From the results obtained the following conclusions were made

1 Permeabilized cells of *K fragilis* possessed better B-galactosidase specific activity than *S thermophilus* and *L delbrueckii* sub sp *bulgaricus*

2 Food grade agar was found to be an efficient and safe immobilizing agent

3 A noticeable reduction in the lactose content was observed within the first half an hour with both the immobilized enzyme system

4 The system could be used efficiently for the hydrolysis of five batches of milk without any reduction in activity A slight brownish discolouration was also noticed on the beads due to long storage of the column

5 Skim milk could be successfully replaced with whey especially lactose hydrolysed condensed whey after adding sufficient quantity of cysteine and yeast extract as a medium for culture maintenance

6 Utilisation of lactose hydrolysed milk for the preparation of products enhanced the activity of starter cultures and reduced the lactose content in the product

significantly making it suitable for consumptions for lactose intolerant individuals

7 Appearance body and texture and flavour scores of products under different treatments revealed that there was no significant difference among them, indicating that they were equally acceptable

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IMMOBILIZATION OF BETA GALACTOSIDASE FOR PRODUCTION OF FERMENTED MILK PRODUCTS WITH LOW LACTOSE

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ABSTRACT OF A THESIS
submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science

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1996

ABSTRACT

An immobilized β-galactosidase enzyme system was developed using permeabilized cells of *K fragilis* as an enzyme source and food grade agar as the immobilizing agent This was utilised for hydrolysing lactose content present in milk which in turn was used for preparing selected fermented products with low lactose content An attempt was also made to assess the possibility of utilisation of whey as a medium for culture maintenance with a view to utilise the by-product

A detailed review of literature has been presented about β-galactosidase specific activity of different organisms various immobilization techniques influence of lactose hydrolysis on physico chemical properties of the product and also about the utilisation of whey as a media for culture maintenance

The experiment comprised of determination of β-galactosidase specific activity of permeabilized cells of three selected organisms and assessing the suitability of agar and sodium alginate as immobilizing agents Since β-galactosidase specific activity was found to be the highest for *K fragilis* it was selected as the best enzyme source Agar was selected as the suitable immobilizing agent because it was found to be safe economical and comparatively more

efficient Using these two raw materials an efficient immobilized enzyme system was developed and its efficiency was assessed by estimating the rate of lactose hydrolysis at fixed time intervals

Selected strains of starter bacteria were screened for their performance in four different media viz skim milk 50 per cent lactose hydrolysed skim milk condensed whey and 50 per cent lactose hydrolysed condensed whey Two media were selected from among the four, which stimulated the starter activity and used for further studies

Three different products viz , yogurt bifidus yogurt and acidophilus milk were prepared using 50 per cent lactose hydrolysed milk obtained by passing through the immobilized enzyme system and cultures maintained separately in lactose hydrolysed milk and lactose hydrolysed whey Two control products were prepared with ordinary milk and above described cultures All the three products under different treatments were analysed for acidity pH tyrosine value total lactic count and sensory evaluation

The results obtained in the study were compared with similar reported findings and the following conclusions were made

S thermophilus and *L delbrueckii* sub sp *bulgaricus*

2 Food grade agar was found to be an efficient immobilizing agent than sodium alginate

3 The immobilized enzyme system prepared with 10 g of *K fragilis* could hydrolyse 50 per cent of lactose content present in 250 ml of milk after holding in the column for four hours at room temperature (30 C)

4 The rate of lactose hydrolysis was found to be the maximum within first half an hour, thereafter a decline in the rate of hydrolysis was observed

5 This system was repeatedly used in five batches without any change in its efficiency or mechanical stability of the beads but after which a reduction in activity was noticed

6 A slight brownish discolouration was observed on the beads when the column was stored for a long period

7 Replacement of skim milk with whey as a starter media slightly enhanced the activity of cultures especially *S thermophilus L lactis* and *B bifidum* whereas *L acidophilus* and *L delbrueckii* sub sp *bulgaricus*

performed better in skim milk especially when the media was subjected to lactose hydrolysis

8 The utilisation of lactose hydrolysed milk for preparation of products slightly enhanced the acidity proteolytic activity and viable cell count of yogurt bifidus yogurt and acidophilus milk

9 In the case of treatment yogurts (Y and Y_2) prepared with lactose hydrolysed milk a significantly higher proteolytic activity was observed

10 Organoleptic evaluation indicated that the utilisation of lactose hydrolysed milk and cultures maintained in lactose hydrolysed skim milk and whey did not affect the flavour and textural characteristics of yogurt On the contrary the flavour as well as body and texture scores of bifidus yogurt and acidophilus milk were slightly improved by this technique