STANDARDISATION AND QUALITY EVALUATION OF BANANA BASED PROBIOTIC FERMENTED FOOD MIXTURES

By

SHARON. C.L

THESIS

Submitted in partial fulfillment of the requirement

for the degree of

DOCTOR OF PHILOSOPHY

(HOME SCIENCE)

Faculty of Agriculture

Kerala Agricultural University

Department of Home Science

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2010

DECLARATION

I, hereby declare that this thesis entitled **"Standardisation and quality evaluation of banana based probiotic fermented food mixtures"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara **Sharon.C.L.**

CERTIFICATE

Certified that this thesis entitled **"Standardisation and quality evaluation of banana based probiotic fermented food mixtures"** is a bonafide record of research work done independently by **Mrs. Sharon.C.L.** under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Dr. V. Usha,
(Major Advisor, Advisory Committee)
Professor and Head
Department of Home Science
College of Horticulture
Vellanikkara

Vellanikkara

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Mrs. Sharon, C.L.**, a candidate for the degree of **Doctor of Philosophy in Home Science** with major in **Food Science and Nutrition**, agree that this thesis entitled **"**Standardisation and quality evaluation of banana based probiotic fermented food mixtures" may be submitted by **Mrs. Sharon, C.L.**, in partial fulfilment of the requirement for the degree.

Dr. V. Usha
(Chairperson, Advisory Committee)
Professor and Head
Dept. of Home Science
College of Horticulture
Vellanikkara

Dr. V. Indira
(Member, Advisory Committee)
Professor
Dept. of Home Science
College of Horticulture
Vellanikkara

Dr. P. Jacob John
Member, Advisory Committee
Professor and Head
Dept. of Processing Technology
College of Horticulture
Vellanikkara

Dr. A. Augustin
(Member, Advisory Committee)
Professor and Associate Director (AR & T)
Kerala Agricultural University
Vellanikkara

Dr.D.Girija
(Member, Advisory Committee)
Professor
Centre for Plant Biotechnology and
Molecular Biology,Vellanikkara

(**EXTERNAL EXAMINER)**

ACKNOWLEDGEMENT

'Feeling gratitude and not expressing it
is like wrapping a present and not giving it.'
(William Arthur Ward)

With immense pleasure, I express my deepest appreciation to my advisor ***Dr. V. Usha,*** *Professor and Head, Department of Home Science, College of Horticulture, Vellanikkara, for her insight, guidance, and motivation, which was always there when I needed it. Her unique way of explaining things in a simple and interesting way, and her willingness to let me follow non-traditional paths made this project an enjoyable experience for me. Her enthusiasm and sound advice always steered me in the right direction. I would certainly like to add that she is one of the best teachers I have ever had.*

I place a deep sense of obligation to ***Dr. V. Indira*** *Professor, Department of Home Science and member of my Advisory Committee for the help and co-operation received from her during the entire programme. She has also offered constructive suggestions for the betterment of this manuscript.*

I am extremely delighted to place on record my profound sense of gratitude to ***Dr. P. Jacob John****, Professor, Department of Processing Technology, for his suggestions and guidance during the conduct of this research work.*

I express my sincere thanks to ***Dr. A. Augustin****, Professor and Associate Director of Research, Kerala Agricultural University, Vellanikkara for his expert advice, kind concern and ever willing help, extended throughout the investigation.*

I also avail this opportunity to pay my sincere thanks to ***Dr. D.Girija ,*** *Professor, Centre for Plant Biotechnology and Molecular Biology, for her fervent interest, cooperation and encouragement.I am deeply indebted to* ***Sri. S. Krishnan,*** *Department of Agricultural Statistics, for his expert advice, constant inspiration with utmost sense of patience and ever willing help rendered in the statistical analysis and interpretations.*

I am genuinely indebted to ***Dr. Chandrasekharan*** *for providing me the needed help during the course of my study.*

Words cannot really express the friendship that I relished from my dear friends ***Aneena*** *and* ***Lakshmy*** *for the sincere help and encouragement, which gave me enough mental strength to get through all tedious circumstances.*

Heartfelt thanks to ***Harish*** *and* ***Suma*** *for thier ever willing help, valuable assistance in the conduct of the microbial work.*

I express my unreserved gratitude and thanks to Bindhya, Kavitha, Mittu, and Saritha who helped me in several ways for the completion of this venture.

My sincere thanks to ***Umaiva chechi*** *for her timely help and cocoperation throughout the study.*

Special thanks go to ***Santhosh chettan,*** *Students Computer Club, College of Horticulture, for rendering necessary help whenever needed.*

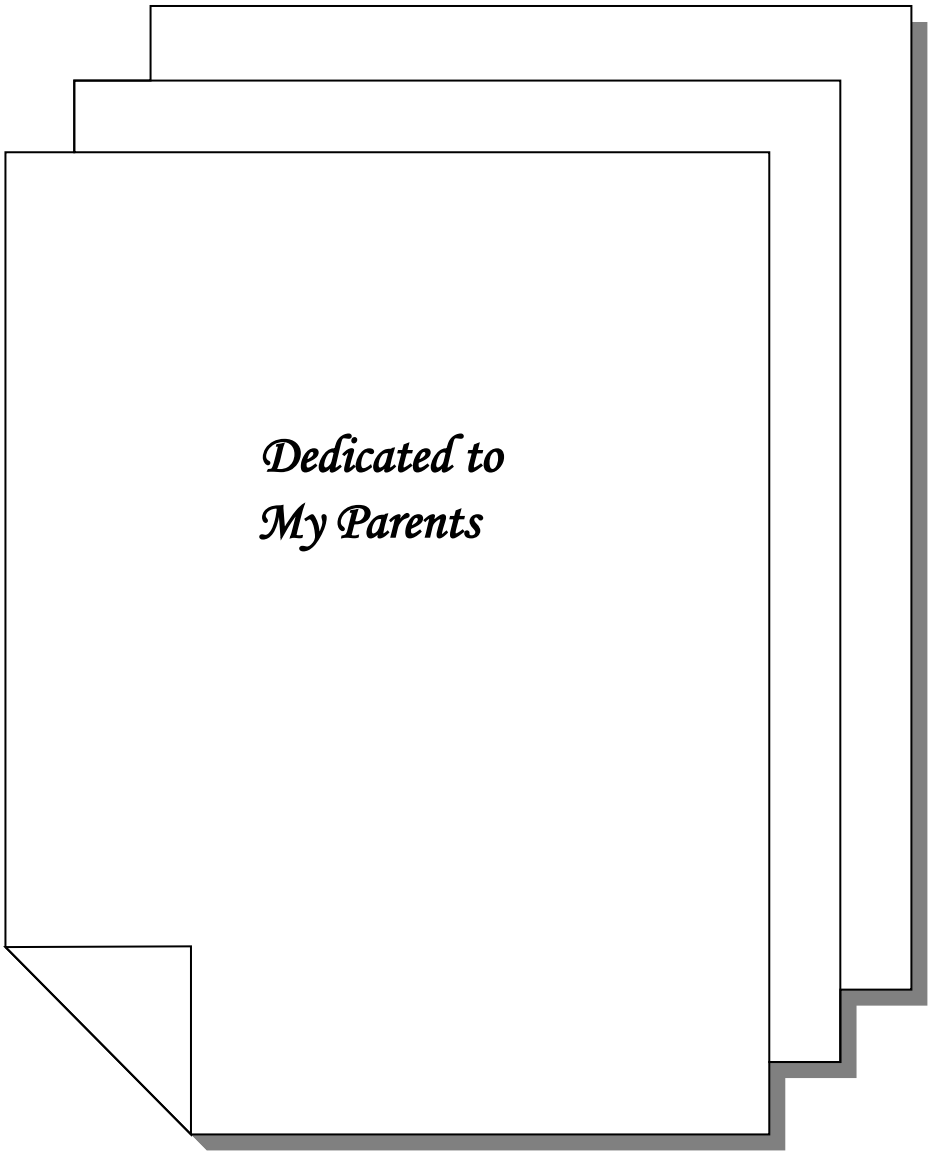
I am thankful to KAU for awarding me the fellowship for the study. I also duly acknowledge ***Dr. Rajeevan****, Associate Dean, College of Horticulture, for allowing me to work as SRF during the course of my study.*

Finally, I am in dearth of words to express my love towards ***My Daddy, Amma and Roshan for*** *their boundless affection, moral support, eternal love, deep concern and prayers which sustains peace in my life. I express my unreserved gratitude to my beloved* ***Husband*** *and my whole extended family members for their affection and encouragement throughout the study.*

Above all, I humbly bow my head before the ***Almighty,*** *who blessed me with will power and courage to complete this endeavour successfully.*

Sharon, C.L.

2004-24-02


Dedicated to
My Parents

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDIX

Abbreviation

cfu	Colony forming units
mm	Millimeter
nm	Nanometer
ppm	Parts per million
g	Gram
kg	Kilogram
mg	Milligram
µg	Microgram
ml	Milliliter
µl	Micolitre
h	Hour/Hours
º C	Degree centigrade
%	Percentage
S	Significant difference
NS	Not significant
IVSD	*In vitro* digestibility of starch
IVPD	*In vitro* digestibility of protein
FFM	Fermented food mixtures
UFFM	Unfermented food mixtures
OAA	Overall acceptability
FC	Fermented control

Introduction

INTRODUCTION

"The doctor of the future will give no medicine, but will interest his patients in the care of human frame, and in the cause and prevention of disease."

- T. A. Edison

The science of human nutrition has moved from a focus on the prevention of nutrient deficiencies to an emphasis on the health maintenance and reduced risk of chronic diseases (Institute of Medicine, 1998).

More recently the focus is on the ability of foods to modulate physiology and biochemistry and thereby confer protection against a range of human diseases. Thus, now we have stepped into an era of 'positive eating' by seeking out foods and food ingredients that offer health benefits.

A variety of foods and their components are emerging as factors capable of modifying growth, development, performance and disease resistance. Such discoveries had influenced perceptions about appropriate nutrition. The term 'functional foods' is coined out of benefits from foods that go beyond those attributable to essential nutrients. Within the concept of functional foods we can identify foods known as 'Probiotics'.

The composition of the diet has a strong influence on intestinal physiology and the metabolism of the microflora. It is well documented that differences in food components, namely proteins, lipids and carbohydrates cause alterations in the composition of bacteria in the gastrointestinal tract. This complex community of microorganisms inhabits the gastrointestinal tract throughout its length. The colon is the main site of microbial colonization and typically, the indigenous microbiota are considered to be made up of more than 500 different species of bacteria (Tuohy *et al*., 2003). Increasing awareness that human intestinal flora is a major factor in health and disease, has lead to different strategies to manipulate the flora to promote health.

At present, it is generally recognized that an optimum balance in microbial population in our digestive tract is associated with good nutrition and health. The microorganisms associated with this balance are *lactobacilli* and *bifidobacteria*. Under natural conditions, the protective gut microflora present is sufficient and there is no need for bacterial supplements. Various factors that call for the need for probiotics are change in food habits, fast life, unhealthy living conditions and excessive consumption of antibacterial substance like antibiotics.

A wide variety of such functional foods are being identified and new products are being developed in which these components are being incorporated. There is an increased interest in these health foods from the consumers seeking alternative approaches to the prevention and treatment of diseases. This increased health consciousness is creating a new market for functional foods.

Increasing number of future functional foods is going to be probiotic derived. Recent advances of research in intestinal flora are the background for the development of probiotic foods. Probiotic foods are such a class of functional foods which have varied health benefits and modulate the GI tract of host organisms (Babu *et al*., 2009).

Factors that negatively influence the interaction between intestinal microorganisms, lead to detrimental effects in health. Increasing evidence indicates that consumption of 'probiotic' microorganism can help maintain such a favourable microbial profile and results in several therapeutic benefits (Khetarpaul, 2005).

Development of foods that promote health and well being is one of the key priorities of food industry (Klaenhammer and Kullen 1999). In recent years, probiotic bacteria have increasingly been incorporated into foods as dietary adjuncts. Several strains of *Lactobacillus paracasei* are used as adjunct cultures added to the cheese to improve flavour development (Antonsson *et al.,* 2003) and to control detrimental microbial activities such as those of *clostridia* and gas forming *lactobacilli*

(Christiansen *et al*., 2005). *L. paracasei* strain reportedly have the potential to survive during heat treatment of cheese (Christiansen *et al*., 2006), supporting the hypothesis that probiotic strains of organism may be investigated for use in other products as well.

While clinical research has progressed, developments have also occurred in the assessment and improvement of the stability of probiotic bacteria in dairy products and functional foods. With their stability, improved and probiotic effect confirmed, we are now approaching the general acceptance of probiotic as documented functional ingredients of foods and beverages.

Using probiotic microorganism, fermented products based on milk or curd have been prepared but much less work have been done on the development of fermented products based on cereals, legumes or other carbohydrate rich flours. There is a considerable interest in extending the range of foods containing probiotic organisms from dairy foods to infant formulae, baby foods, fruit juice based products, cereal based products and pharmaceuticals (Donohue and Salminen, 1996).

A staple based food mixture if developed from the commonly used foods in a community and then fermented with probiotic organism, it may have a better profile of nutrients, acceptability and therapeutic value. Hence, the present study entitled "Standardisation and quality evaluation of banana based probiotic fermented food mixtures" was undertaken with the following objectives

1. To standardise indigenous food mixtures based on banana flour with probiotic fermentation involving *Lactobacillus acidophilus*

2. To evaluate the nutritional factors, organoleptic qualities and storage stability of food mixtures

Review of literature

2. REVIEW OF LITERATURE

"*Let food be thy medicine and medicine be thy food*", the age old quote by Hippocrates is certainly the tenet of today. With the growing interest in self- care and integrative medicine coupled with our healthy embracing baby boomer population, recognition of the link between diet and health has been stronger. As a result, the market for foods that promote health beyond providing basic nutrition is flourishing. According to Osawa (1998), the primary function of food is to provide essential nutrients, the secondary function is to satisfy sensory attributes and to prevent diseases at the molecular level is the tertiary function. Viewing such an importance linked with food, the concept of functional food has been visualized. Within the concept of functional foods we can identify foods known as 'Probiotics'. The consumer's overwhelming interest for functional foods, including probiotics, make it imperative that health professionals stay abreast of the latest research findings and available products. This chapter contains the review on the following heads:-

2.1. FUNCTIONAL FOODS

"*An apple a day keeps the doctor away*" could perhaps be considered the first functional food advertisement.

A variety of foods and their components emerging as factors capable of modifying growth, development performance and disease resistance, that go beyond those attributable to essential nutrients and the foods that contain significant levels of biologically active components that impart health benefits beyond basic nutrition are generally referred to as functional foods (Sanders, 1998). These foods contain adequate amount of one or a combination of components which affects the functions in the body so as to have positive cellular and physiological effects (Roberfroid, 1998). Functional foods are said to be one step ahead of healthy natural foods in assisting the therapeutic process of the body towards substitution of medicines (Diplock *et al*., 1999).

Functional foods-also known as designer foods, medicinal foods, therapeutic foods, super foods, foodiceuticals and medifoods are defined as foods that contain some health promoting components beyond traditional nutrients (Berner and O'Donnel, 1998).

A functional food should be a food derived from natural food ingredients, which can be consumed as a part of daily diet. It should perform certain body functions like enhancement of body's natural defense mechanism, prevent and ensure rapid recovery from specific diseases, control physical and mental conditions and slow down the ageing process (Chaturvedi, 2001).

According to Varshey (2002), a dietary ingredient that affects its hosts in a targeted manner so as to exert positive effects (so as to justify a health claim) can be classified as functional ingredient.

The functional foods comprise (i) conventional foods containing naturally occurring bioactive substances (eg: dietary fibre) (ii) foods enriched with bioactive substances (eg: probiotics, antioxidants) and (iii) synthesised food ingredients introduced to traditional foods (eg: prebiotics) (Grajek *et al.,* 2005).

The development of functional foods products will continue to grow well in the 21st century as consumer demand for these products are heightened and the market for these foods is growing at a rate of 15-29% per year and the industry is claimed to be worth $ 33 billion (Hilliam , 2000)

Pisulewski and Kostogrys (2003) pointed out that in the industrialized world there has been an explosion of consumer interest in functional foods for the well being, and life prolongation as well as in the prevention of initiation, promotion and development of cancer, cardiovascular diseases and osteoperosis.

The Global market size of functional foods has been estimated between US$ 30 and US$ 60 billion depending on the definition, with Japan, the United States, and Europe as the biggest markets and the developing countries have started to emerge as exporters to cater to the increasing demand in the developed countries (Williams *et al*., 2006). According to a recent report in the Datamonitor (Douad, 2007) probiotic drinks and yoghurts are the leading functional foods with market growth.

2.2. DEFINITION OF PROBIOTICS

"Food-Friendly Bugs"

The origin of the term ‘probiotic’ is credited to Werner Kollath who proposed the term ‘Probiotika’ to designate ‘active substances that are essential for a healthy development of life’ which is related in a publication by the German scientist, Vergin (1954).

The term ‘probiotic’ meaning ‘for life’ is derived from the Greek language and it was first used by Lilly and Stillwell (1965) to describe substances produced by one protozoan that stimulated the growth of another. Parker in 1974 used the term to describe organisms and substances which contribute to intestinal microbial balance.

However, the term probiotics was popularised by Fuller (1989) who argued that the latter definition is too imprecise, since substances mentioned would include antibiotics and later revised the definition as live microbial food supplement which beneficially affects the host by improving its intestinal microbial balance. A quite similar definition was proposed by Huis and Havenaar (1992) for probiotics as ‘a mono or mixed cultures of live microorganisms which when, applied to man as a fermented product, affects beneficially the host by improving the properties of the indigenous microflora.

Another, but probably not the last definition is, probiotics are selected viable microbial dietary supplements that when introduced in sufficient quantities, benefically affect human oraganism through their effects in intestinal tract (Dimer and Gibson, 1998; Ziemer and Gibson, 1998; Sanders 1998; Guarner and Schaafsma, 1998; Vaughan *et al*., 1999 and Zubillaga *et al.,* 2001).

The most recent definition was by Schrezenmeir and De Vrese (2001). They defined probiotics as viable microbial food supplements which beneficially influence

the health of the host. These microorganisms interact with the diet and the host, contributing to protection against intestinal pathogens through colonisation resistance and providing nutritional and health benefits via their metabolic activities (Guarner and Malagelada, 2003).

2.3. PROBIOTIC MICROORGANISMS

Probiotics can be bacteria, moulds or yeast, among which *Lactobacilli, Streptococci* and *Bifidobacteria* are the commonly used groups in the production of probiotics. The justification for the use of *Lactobacilli* stems from studies which show that when the gut flora develops afterbirth, as *Lactobacilli* increases, other components of the flora decrease (Smith, 1965).

The organisms as species used in probiotic preparation include *Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casie, Lactobacillus lactis, Lactobacillus helviticus, Lactobacillus salivarius, Lactobacillus rhamnosus, Lactobacillus johnsonii, Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus delbrueckii, Lactobacillus gasseri, Lactobacillus plantarum, Bifidobacterium bifidum, B.longum, B.breve, B.infantis, B.lactis, B.aadoescentis* and *Escherichia coli* (Krishnakumar and Gordon, 2001 and Heyman and Menard, 2002). Some other species or other microorganism used are *Streptococcus thermophillus, Enterococcus faecium, Enterococcus faecalis, Sacchromyces boulardi, Sacchromyces cerevisiae, A.niger* (Suvarna and Boby, 2005) and *A.Orizae and C.Pintolepesii* (Anuradha and Rajeshwari, 2005). Majority of the probiotics are bacterial (gram positive) (Khetarpaul, 2005).

According to Oyetayo and Oyetayo (2005) the following terms can be used to distinguish different probiotic microorganisms:

Research strain: This is microorganism generally regarded as safe (GRAS) being studied for probiotic application, but not commercially available in any market

Commercial strain: A strain produced on an industrial scale for commercial use, as a fresh product (fermented milk, juice etc) or nutritional supplement (capsules or sachets).

Probiotic strain: Any micro organism, generally regarded as safe (GRAS) (such as *Lactobacilli*, *Bifidobacteria*, *Streptococci, Saccharomyces,* etc) shown in published research to have one or more of the following positive attributes: *In vitro* adherence to epithelial cells, *in vitro* antimicrobial activity, *in vitro* resistance to bile, hydrochloric acid, and pancreatic juice, anticarcinogenic activity (reduction of carcinogens) in clinical trials, immune modulation or stimulation in clinical trials, reduction of intestinal permeability in clinical trials and colonisation in the GIT in clinical trials.

Implantable strain: Any microbial strain native to the GIT of man (that is, *Lactobacilli* or *Bifidobacteria*) shown to survive passage through the GIT (appear live in stool) or persist on biopsies of the GIT mucosa after cessation of feeding.

Clinical strain: An implantable strain which has been shown to have one or more specific health benefits, and therefore have demonstrated clinical usefulness. Some examples of benefits that have been shown are reduced intestinal permeability, enhancement of immune functions, and treatment of infection.

2. 4. CHARACTERISTICS AND FEATURES OF A GOOD PROBIOTIC

A good probiotic should be a strain, which is capable of exerting a beneficial effect on the host animal (e.g. increased growth or resistance to disease). It should be non-pathogenic and non-toxic, should be present as viable cells, preferably in large numbers, should be capable of surviving and metabolising in the gut environment (e.g. resistance to low pH and organic acids), it should be stable and capable of remaining viable for periods under storage and field condition and the strain should be safe and tested for human use (Fuller, 1989).

The survival of probiotic organisms in the gut depends on the colonization factors that they possess, organelles which enable them to resist the antibacterial mechanisms that operate in the gut and need to avoid the effects of peristalsis (which tend to flush out bacteria with food) which can be achieved either by immobilising themselves or by growing at a much faster rate than the rate of removal by peristalsis and the strains need to be resistant to bile acid (Seo *et al*., 1989).

Probiotics generally enhance the intestinal microflora by replenishing suppressed bacteria and inhibiting the growth of pathogenic flora (Salminen and Deighton, 1992).

According to Coconnier *et al.* (1992) the ability of probiotic bacteria to adhere to the intestinal cell wall is an important prerequisite for colonization in the gastro intestinal tract.

According to Clark *et al*. (1993) the acid and bile tolerance is strain dependent and Lankhaputra and Shah (1995) showed that *Bifidobacteruim longum* survives better in acidic conditions and is able to tolerate a bile concentration as high as 4 percent.

One of the most important criteria for selection of a probiotic organism is their ability to survive in acidic environment of the product and in the stomach, where the pH can reach as low as 1.5 and the organism must be able to survive in these bile concentrations (Lankhaputhra and Shah, 1995).

It is also important that probiotic strains to be antagonistic against carcinogenic and pathogenic bacteria either by antimicrobial substances production or competition exclusion and supporting this, Dave and Shah (1997) reported that lactic acid bacteria produce hydrogen peroxide, diacetyl and bacteriocin as antimicrobial substances which create hostile environments for food borne pathogens and spoilage organisms.

According to Lankaputhra and Shah (1998) only one out of six strains of lactobacillus adhered properly whereas two out of nine strains of bifidobacterium showed good adherence properties and in general bifidobacterium spp adheres better than *L .acidophilus*.

2. 5. BENEFICIAL EFFECTS OF PROBIOTIC ORGANISMS

The concept of probiotics was evolved around 1900. At this time Henry Tissier, a French Paediatrician, observed that children with diarrhoea had in their stools a low number of bacteria characterised by a peculiar, Y shaped morphology. These "bifid" bacteria were, on the contrary, abundant in healthy children (Tissier, 1906).

Nobel price-winner, Metchinkoff (1908) advocated the consumption of *Lactobacilli* in controlling endogenous intoxication (autointoxication) caused by wrong types of components in the intestinal flora. He pointed out that the long, healthy lives of Bulgarian peasants were the result of their consumption of fermented milk products. The works of Metchnikoff and Tissier were the first to make scientific suggestions about the probiotic use of bacteria.

The beneficial effects of probiotic will depend on a number of factors including the strain chosen, level of consumption, duration and frequency of exposure, and the physiological condition of the individual (Koop, 2001).

2.5.1. Anticarcinogenic activity

Probiotics have been extensively studied under *in vitro* and *in vivo* conditions and have been well documented to have antitumour activities due to inhibition of tumour cells and destruction of carcinogens (Fuller, 1989).

The antitumour action of probiotics may be due to (i) inhibition of carcinogens and /or procarcinogens (ii) inhibition of bacteria that convert procarcinogens to

carcinogens, (iii) activation of host immune system, (iv) reduction of intestinal pH to reduce microbial activity and (v) alteration of colonic motility and transit time (Mclntosh, 1996). Probioitics lowers the intestinal pH, create a bactericidal environment and modulate the bacterial enzymes (Lee and Salminen, 1995). The Administration of *Lactobacilli* and *Bifidobacteria* modify the flora, leading to decreased β glucoronidase and carcinogen levels (Hosada *et al.*, 1996).

Aso and Akazan (1992) has reported that in both experimental animals and humans, probiotic consumption has reduced the risk of colon cancer by reducing the incidence and number of tumours and the experimental results suggested that the consumption of *L. casei* delayed the recurrence of bladder tumours.

Reddy *et al.* (1993) have reported that feeding yoghurt to swiss mice resulted in 25-35 % reduction in Ehrlich ascites tumour cells when compared to control groups fed milk.

Okawa *et al*. (1993) stated that the mechanism of tumor suppression may involve a role of *B.longum* as an immunomodulator and biological response modifier.

The colonizing cells of *Bifidobacterium* produce lactic acid and lower the intestinal pH and create an unfavourable bacterial environment for the enteropathogens and thus develop a favourable microenvironment which modulates the bacterial enzymes (Sekine *et al*., 1994).

Morotomi (1996) has reported that *L.casei shirota* strain as a lactic acid bacterium has increase potential for cancer chemoprevention

Fermented milk and yoghurt when consumed have shown to lower the incidence of colon cancer or lower propensity to develop large adenocarcinomas (Bourtan, 1996).

Consumption of probiotic bacteria with oligosaccharide could promote bacterial growth in the colon and hence produces greater quantities of short chain fatty acids such as butyrate, which has been shown to have antitumour effects at the cell level (Young, 1996). But there is insufficient evidence that the benefits of probiotics, such as prevention of colon cancer are exerted through short chain fatty acids (Topping, 1996).

Some probiotic bacteria produce butyric acid and this molecule can influence the rate of apoptosis in enterocytes and also act as an anticarcinogen by neutralizing the activity of mutagens such as 4-nitroquinoline-n-oxide, 2-nitroflurorene and benzopyrene (Salminen *et al*., 1998a).

Oatley *et al*. (2000) reported that *in vitro* studies with *L. rhamnosus GG* and *Bifidobacteria* and *in vivo* study using *L. rhamnosus GG* and *LC-705* and propioni bacterium spp showed a decrease in availability of carcinogenic aflatoxin in the lumen.

According to Parvez *et al*. (2006) probiotc cultures decrease the exposure to chemical carcinogens by producing compounds that inhibit the growth of tumor cells by stimulating the immune system.

2. 5.2. Cholesterol and risk of cardiovascular disease

The use of probiotic *Lactobacilli* and metabolic byproducts potentially confer health benefits to the heart, including prevention and therapy of various ischemic heart syndromes (Oxman *et al*., 2001) and lowering serum cholesterol (De Roos and Katan, 2000).

Mann and Spoerry (1974) observed a decrease in serum cholesterol levels in men fed large quantities of milk fermented with *Lactobacillius* and this may have been due to the production of hydroxymethyl glutarate by lactic acid bacteria which inhibit hydroxymethyl glutaryl CoA reductases required for the synthesis of cholesterol. In

another study Mann (1977) concluded that consumption of large quantities of cultured yoghurt lowered serum cholesterol levels in human volunteers.

Rao *et al*. (1981) reported that metabolites from orotic acid formed during fermentation of dairy products may help lower cholesterol levels. They conducted experiments and reported that liver cholesterol levels were lower in the group receiving thermophilus milk than the group receiving skim milk

According to Jaspers *et al*. (1984) uric acid produced inhibits cholesterol synthesis and orotic acid and hydroxymethyl glutaric acid reduce serum cholesterol.

Gilliland *et al.* (1985) showed that *L. acidophilus* itself may take up cholesterol during growth in the small intestine and make it unavailable for absorption into the blood stream

Honma (1988) in his study reported that feeding of fermented milks containing very large numbers of probiotic bacteria (- 10^{9} /g) to hypercholestemic human subjects lowered cholesterol levels from 3.0 to 1.5 g /L.

Klaver and Meer (1993) reported that removal of cholesterol from the culture medium by *L. acidophilus* is due to the deconjugation of bile acids. By deconjugation, bile acid will not adsorb lipid as readily as the conjugated counterpart, leading to a reduction in cholesterol level.

Fukushima and Nakano (1996) compared the effect of a mixture of probiotic organisms *(Bacillus, Lactobacillus, Streptococcus, Clostridium, Sacchromyces and Candida*) on lipid metabolism with that of *L. acidphilus* and *S. faecalis* and concluded that total serum cholesterol concentration of the group fed on the mixture organism decreased by 15-33 per cent compared with the other groups at the end of 4 week feeding period.

Schaafsma *et al*. (1998) reported that adult male volunteers fed with 125ml *L. acidophilus* fermented milk , three times daily for 3 weeks showed significantly lower values for serum total cholesterol, LDL cholesterol and LDL/HDL ratio by 4.4,5.4 and 5.3 per cent respectively with no change in the levels of serum HDL cholesterol, triglycerides and blood glucose.

Pearce, 1996 and Anderson and Gilliland, 1999 found that intake of about one cup of yoghurt with live cultures per day for one year prevented an increase in blood total and LDL cholesterol levels in adults. According to James *et al*. (1999) fermented milk containing *L. acidophilus* L1 was accompanied by a 2.4 per cent reduction of serum cholesterol concentration (200 ml of fermented milk daily for 3 weeks).

Lin and Chen (2000) investigated cholesterol reducing abilities of 6 strains of *L. acidophilus* and found that *in vivo* hyporcholesterolemic ability is likely due to the assimilation of cholesterol by *L. acidophilus* cells or/ and attachment of cholesterol to the surface of *L. acidophilus* cells.

Preliminary studies of Nakamura *et al.* (1995) indicated that probiotic bacteria or their fermented products may also play a role in blood pressure control with animal and clinical studies documenting antihypertensive effects of probiotic ingestion. Elderly hypertensive patients who consumed fermented milk with a starter containing *Lactobacillus helveticus* and *Saccromyces cervisiae* experienced reductions in systolic and diastolic blood pressure (Hata *et al*., 1996).

2.5.3. Ulcer

Probiotic bacteria suppress the growth of *Helicobacter pylori*, which is known to cause peptic ulcer (Lambert and Hull, 1996). A *L. acidophilus* strain was reported to secrete an antibacterial substance against *H. pylori* which decrease the adhesion and viability of *H. pylori* (Coconnier *et al*., 1997). *In vitro* and animal data indicate that lactic acid bacteria can inhibit the growth of the pathogen and decrease urease enzyme

activity necessary for the pathogen to remain in the acidic environment of stomach (Midolo *et al*., 1995; Kabir *et al*., 1997 and Aiba *et al*., 1998).

Michetti *et al*. (1999) observed the inhibition of *H. pylori* infection in humans consuming *L. johnsonii*. Probiotics was proposed for use in the treatment of *H. pylori* infection for improving eradication rate and permeability and for compliance of multiple antibiotic regimens used for the infection (Bazzoli *et al*., 1992 and Filippo *et al*., 2001). In a study on *H. pylori* infected volunteers, acidified milk containing *L. johnsonii* showed to decrease *H. pylori* density and reduce inflammation in the antrum (Felley *et al*., 2001).

L .gasseri was shown to suppress *H. pylori* and reduces mucosal inflammation (Sakamoto *et al.,* 2001).

Macfarlane and Cummings (2002) observed some preliminary evidence that probiotic bacteria may inhibit the gastric colonization and activity of *H. pylori*, which is associated with gastritis, peptic ulcer and gastric cancer.

Lactic acid bacteria are often able to survive the acidic gastric conditions and show beneficial influence in eradication of *H. pylori*, which is involved in the process of gastric ulcer development (Khetarpaul, 2005).

2.5.4. Allergy

Probiotics by their potential effect on the non-immunologic and immunologic defense barrier of the gut, alleviate the inflammatory response in food allergy. *Bifidobacteria* and *Lactobacilli* have shown to enhance IgA production in Peyer's patches and potentiate IgA response to potentially harmful antigens (Yasui *et al.,* 1992). Trapp *et al*. (1993) reported that administration of probiotics was associated with disappearance of food allergy manifestation with decrease in concentration of IgE in the

serum and with lower frequency of allergies. Probiotics induce the secretion IL-12 which increases the resistance to intracellular bacteria and parasites (Trinchieri, 1994).

Lactobacillus GG and other *Lactobacilli* are reported to hydrolyse the complex casein (that trigger the first allergic reaction in some milk fed infants) to smaller peptides and amino acids and hence decrease the proliferation of mitogen induced human lymphocytes (Sutas *et al*., 1996). **A**ccording to Majamaa and Isolauri (1997), probiotics such as *Lactobacillus GG* exert a beneficial effect on allergic reaction by improving the mucosal barrier function and alleviate the symptoms as those associated with milk protein.

Isolauri *et al*. (2000) in their study with infants allergic to cow's milk concluded that atopic dermatitis was alleviated by ingestion of probiotic strains, *L. rhamnosus GG* and *B. lactis BB-2*. This is based upon the ability of *Lactobacilli* to reverse increased intestinal permeability, enhance gut-specific IgA responses, promote gut barrier function, thorough restoration of normal microbes and enhance transforming growth factor beta and interleukin production as well as cytokins that promote production of IgE antibodies (Isolauri, 2001).

Probiotics have been shown to reduce the incidence of childhood eczema by half, compared to placebo, when administrated during pregnancy upto 6 months postnatally (Kalliomaki *et al.,* 2001). A follow up study by Rautava *et al.* (2002) demonstrated a two fold increase in transforming growth factor B2, an anti inflammatory cytokines, in the breast milk of mothers receiving probiotics compared to placebo. Moreover, there was a reduction in the risk of atomic eczema in children whose mother's received probiotics compared to placebo (15 versus 47%). Kirjavainen *et al.* (2003) reported that supplementation of infant formulae with viable LGG is a potential approach for the management of atomic eczema and cows milk allergy.

2.5.5. Lactose intolerance

Birge *et al.* (1967) confirmed that when probiotics are fed to patients with lactose tolerance, milk lactose is hydrolysed by probiotic strains and lactose is assimilated and calcium absorption is also favoured. Kim and Gilliland (1983) found that feeding fermented milk to lactose intolerant subjects results in a significantly lower level of hydrogen (lower hydrogen level indicates that lactose has been metabolized prior to entering the large intestine) in the breath when compared to the level for subjects fed unfermented milk.

The increased tolerance for dairy products containing probiotic cultures could be due to intra-intestinal digestion of lactose by β d- galactosidase released from the cultures (Saviano *et al.,* 1984).

Intake of yoghurt improves tolerance to milk in individuals with cow's milk allergy (Kolars *et al.,* 1984). Onwulata *et al*. (1989) have reported the effects of acidophilus milk in alleviating lactose malabsorption. The beneficial effect appears to be a consequence of the lactic acid bacteria in fermented milk increasing latase activity in the small intestine (Marteau *et al*., 1990 and Pelletier *et al*., 2001).

Probiotic yoghurt is tolerated well by lactose malabsorbers since some lactose is hydrolysed by yoghurt bacteria during fermentation and also may be coagulated milk, because of its viscous nature may pass slowly through the gut than unfermented milk (Shah *et al*., 1992).

Fresh yoghurt is more sufficient in facilitating lactose digestion than heated yoghurt. The β galactosidase activity in yoghurt drops by 80 per cent in the duodenum, one fifth of the yoghurt lactase activity is still found in the terminal ileum, suggesting a relative persistence of protein along the digestive tract. The bacterial β galactosidase present in yoghurt is partly resistant to luminal hydrolysis but it can hydrolyse lactose in

the mid and distal part of the small intestine where the pH is compatible with the enzymatic activity (Shermak *et al*., 1995).

S. thermophilus, L. bulgaricus and other *Lactobacilli* in fermented milk products can alleviate symptoms of lactose intolerance by providing bacterial lactase to the intestine and stomach (Dairy council of California, 2000).

According to Khetarpaul (2005) yoghurt tolerance is mainly due to supply of lactase activity from lactic acid bacteria present in yoghurt and the bacteria must be live and present in sufficient quantity to exert the beneficial effect (Yoghurt containing 10^{8} bacteria /ml are required).

2.5.6. Diarrhoea

Administration of *Lactobacillius GG* (LGG) has been shown to stop episodes of relapsing diarrhoea caused by a toxin producing strain of *Clostridium difficle* (Alm, 1983). Cultured milk containing *L. acidophilus, S. thermophilus and B. longum* was administered to demented senile patients with diarrhoea who habitually used purgatives and were shown that the stool frequency decreased and their condition relating to diarrhoea improved as well as significant increase in the number of *Bifidobacteria* in their faeces (Takiguchi *et al*, 1985). Gorbach *et al*. (1987) demonstrated that *lactobacillus GG* successfully eradicated *C. difficle* in five patients with relapsing colitis when they were fed viable *Lactobacilli* in skimmed milk, daily. According to Siitonen *et al*. (1990) volunteers with diarrhea, who received the probiotic with erythromycin showed improvement than those who consumed pasteurized yoghurt as control

Probiotc consumption is found to be useful in the treatment of many types of diarrhoea, including antibiotic associated diarrhoea in adults, traveler's diarrhoea and diarrhoeal diarrhoea in young children caused by *rotaviruses* (Oksanen *et al.,* 1990 and Isolauri *et al.*, 1991).

Lactobacillus GG has been shown to lower the rate of diarrhoea in Finnish people traveling to Turkey (Oksanen *et al.*, 1990) and Americans traveling to developing countries (Hilton *et al.*, 1997).

An enhancement of the circulating IgA antibody secreting cell response was observed in infants supplemented with a strain of *L. casei* and was related to prevention of diarrhoea in the study group compared with a control group (Kaila *et al*., 1992).

Saavedra *et al.* (1994) showed that supplementation of infant milk formula with *B. bifidum* and *S. thermophilus* reduced *rotavirus* shedding and episodes of diarrhoea in children. Biller *et al.* (1995) reported that administration of probiotics can alleviate the signs and symptoms of *C.difficle* infection. *Lactobacillus rhamnosus GG* and *Bifidobacterium BB-12* are found beneficial for the prevention and treatment of acute diarrhoea mainly caused by rotavirus in children (Guandalini *et al*., 2000 and Szajewska *et al.*, 2001).

Probiotics have proved useful as a prophylactic regimen and potentially they can also be used to alleviate the signs and symptoms of antibiotic induced diarrhoea (Armuzzi *et al.,* 2001).

2.5.7. Immune system stimulation

An enhancement in the non specific immune phagocyte activity of granulocyte populations in the blood of human volunteers after consumption of *L. acidphilus* and *B. bifidum* has been documented by Schiffrin *et al*. (1995). Probiotics provides immune enhancement either by absorption of a soluble antigen or by translocation of *Lactobacilli* through the gut wall into the blood stream (Schiffrin *et al.,* 1997).

Ingestion of probiotic yoghurt has been reported to stimulate cytokines production in blood cells (Solis and Lemonnier, 1996) and enhance the activity of macrophages (Morteau *et al.*, 1997). Gill (1998) has reported that lactic acid bacteria

excert their immunity enhancing effects by augmenting both non specific and specific host immune responses.

Biologically effective probiotic bacteria within the intestinal lumen exert their influence by inhibiting the growth of enteric pathogens through the production of lactic acid and antimicrobial peptides, known as bactriocins. Attachment of probiotic bacteria to receptors on the cells surface of intestinal epithelial cells can activate signaling process, leading to the synthesis of cytokines that affect the function of mucosal lymphocyte (Salminen *et al*., 1998b). Ingestion of probiotic yoghurt has been reported to stimulate cytokins production in blood cells (Solis and Lemonnier, 1996) and enhance the activity of macrophages (Morteau *et al*., 1997).

According to Sanders (1998), Donnet-Hughes *et al.* (1999) and Perdigon *et al.* (1999) both specific and non specific immune response by activating macrophages, increased the levels of cytokines, increasing natural killer cell activity and increasing levels of immunoglobulins.

According to Mack *et al*. (1999) and Miettinen *et al.* (2000) there is an induction of mucus production or macrophages activation by *Lactobacilli*, signaling stimulation of IgA and neutrophils at the site of probiotic action (gut) .

Intravenous, intraperitoneal and intrapleural injection of *L. casei shirto* into mice significantly increased natural killer activity of mesenteric node cells but not of Peyer's patch cells of spleen (Matsuzaki and Chin, 2000), supporting the concept that some probiotic strains can enhance the innate immune response.

A number of studies have been performed *in vitro* and in animals (Gill *et al.*, 2000) which clearly showed that probiotic strains can modify immune parameters. In a series of randomized, double blind, placebo controlled clinical trials, it was demonstrated that dietary consumption of *B. lactis HN019* and *L. rhamnosus HN001* resulted in measurable enhancement of immune parameters in the elderly (Arunachalam *et al*., 2000, Shah *et al.,* 2000 and Gill *et al*., 2001).

2.5.8. Irritable bowel syndrome

Probiotic bacteria have shown to preserve intestinal integrity and mediate the effects of irritable bowel syndrome of colitis and alcoholic liver disease (Nanji *et al*., 1994 and Kruis *et al*., 1997).

Gionchetti *et al.* (2000) and Gupta *et al.* (2000) have reported the potential role of probiotics in therapy and prophylaxis and that combination of strains have a role to play in remediation.

Neidzielin *et al.* (2001) studied the efficacy of *Lactobacillus plantarum299V* and reported that it seems to have a beneficial effect in patients with irritable bowel syndrome.

A probiotic formulation 'VSL' appears to be promising in the relief of abdominal bloating in patients with diarrhoea-predominant irritable bowel syndrome (Kim *et al*., 2003). A similar study was conducted by Kajander *et al.* (2005) with a probiotic mixture containing *Lactobacillus rhamnosus* GG, *L. rhamnosus* LC705, *Bifidobacterium breve* Bb99 and *Propionibacterium freudenreichii* ssp. *shermanii* JS and was found effective in alleviating irritable bowel syndrome symptoms.

2.5.9. Inflammatory bowel disease

Encouraging data have already been obtained in experimental murine models, saying *lactobacillus* strains have been effective in patients with inflammatory bowel disease (Collins *et al*., 1998 and Madsen *et al*., 1999).

Probiotics by their immunomodulatory and bowel flora manipulating properties, show a promising effect in the treatment of chronic inflammatory bowel disease (Schultz, 2000).

Controlled clinical studies have demonstrated the efficacy of probiotics in the maintenance of remission of pouchitis, prophylaxis of pouchitis after the formation of an ileoanal reservoir, maintenance of remission of ulcerative colitis, and treatment of Crohn's disease (Hart *et al*., 2003 and Kruis, 2004).

2.5.10. Vaccine adjuvants

Among the various potential health benefits of lactic acid bacteria include the use as an oral adjuvant (Perdigon *et a.l*, 1995).

Isolauri *et al*. (1995) noted an increase in rotavirus specific IgM secreting cells when the children were given *lactobacillus GG* as an adjuvant to an oral vaccine to rotavirus compared to placebo on 8^{th} post vaccination day.

HeFang *et al*. (2000) conducted a study in which thirty healthy volunteers were randomised into three different treatment groups and consumed *Lactobacillus* GG, *Lactococcus lactis* or placebo (ethyl cellulose) for 7 days and on days 1, 3 and 5, an attenuated *Salmonella typhi* Ty21a oral vaccine was given to all subjects to mimic an enteropathogenic infection. The result showed that a greater increase in specific IgA among the subjects receiving the vaccine in combination with *Lactobacillus* GG and those receiving *L. lactis* with their vaccine evinced significantly higher CR3 receptor expression on neutrophils than those receiving either the placebo or *Lactobacillus* GG. These results indicate that probiotics may influence differently the immune response to oral *S. typhi* vaccine and that the immunomodulatory effect of probiotics is strain-dependent.

It is shown that an oral administration of the strain *L. fermentum* CECT5716 potentates the immunologic response of an anti-influenza vaccine and may provide enhanced systemic protection from infection by increasing the T-helper type 1 response and virus-neutralizing antibodies (Olivares *et al.,* 2007).

2.5.11. Urogenital tract infection

The dominant presence of *Lactobacilli* in the urogenital microflora of healthy women and the obliteration of *Lactobacilli* in patients who develop urogenital tract infection (Stamey, 1973 and Schaeffer and Stamey, 1977), bacterial vaginosis, and many other genital infections (Hillier *et al*., 1993) [except candidiasis]has led to a focus on these bacteria

A 1992 study published in the Annals of Internal Medicine suggests that *L. acidophilus* may reduce the recurrence of vaginal infections caused by *candida* (Hilton and Isenberg, 1992). In fact, eating yoghurt has long been an "alternative" treatment for yeast infections.

Daily oral intake of probiotic strains *Lactobacillus rhamnosus GR-1* and *Lactobacillus fermentum RC-14* resulted in asymptomatic bacterial vaginosis patients reverting to normal *Lactobacilli* dominated vaginal microflora (Reid *et al.,* 2001 and Reid *et al.*, 2003).

2.5.12. Kidney stones

Sidhu *et al*. (2001) reported that rats with chronic hyperoxaluria resulting from high dietary oxalate that were treated with *O. formigenes* showed decreased urinary oxalate within 2 days of initiating probiotic supplementation and the approach may be feasible for treating calcium oxalate kidney stone disease.

Treating patients or preventing stone formation with bacteria may be an effective way of reducing their risk of repeatedly developing painful kidney stones. A study by Kaufman *et al*. (2008) revealed that *Oxalobacter formigenes*, a Gram-negative, anaerobic bacteria as a probiotic, can break down oxalate in the intestinal tract. This bacterium is present in many normal adult people. The authors concluded through this

study that colonization with *O. formigenes* is associated with a 70 percent reduction in the risk for recurrent calcium oxalate stone formation.

2.5.13. Liver cirrhosis

Patients with liver cirrhosis have an imbalance of intestinal bacterial flora and probiotics effectively increase the *Bifidobacterium* count and reduce the risk (Zhao *et a.l,* 2004).

Modulation of intestinal flora through the use of probiotics is an emerging therapeutic strategy in the management of chronic liver diseases (Sheth and Garcia-Tsao, 2008).

In an open-label study by Stadlbauer *et al*. (2008) patients with alcoholic cirrhosis ($n = 12$) received *Lactobacillus casei* Shirota (6.5×10^9) 3 times daily for 4 weeks and the data were compared to healthy controls ($n = 13$) and cirrhotic patients ($n = 8$) who did not receive probiotics and the result revealed that the probiotics restore neutrophil phagocytic capacity in cirrhosis, possibly by changing IL10 secretion and TLR4 expression.

2.5.14. Hepatic encephalopathy

Probiotics have multiple mechanisms of action that could disrupt the pathogenesis of hepatic encephalopathy and may make them superior to conventional treatment (Solga, 2003).

Probiotic supplementation in the form of probiotic yoghurt to non alcoholic minimal hepatic encephalopathy cirrhotics for 60 days showed a significant rate of minimal hepatic encephalopathy reversal, and excellent adherence in cirrhotics (Bajaj *et al.*, 2008).

2.5.15. Constipation

A lower pH enhances peristalsis of the colon and subsequently decreases colonic transit time which is beneficial in the treatment of constipation (Picard *et al.*, 2005 and Bouvier, 2001).

Probiotic strains, such as *L. shirota* and the *B. infantis*, increase defecation frequency and soften stools in adults with constipation and *IBS* (Koebnick *et al*., 2003 and Whorwell *et a.l,* 2006). A recent study in children with constipation showed an increase in defecation frequency and a decrease in abdominal pain using the strain *L. rhamnosus* (Bu *et al.,* 2007).

A non randomised non placebo controlled pilot study by Bekkali *et al.* (2007) on 20 constipated children, on a daily probiotic supplement containing a mixture of *Bifidobacteria bifidum, B. infantis, B. longum, L.casei, L. plantarum and L. rhamnosus.,* showed to boost the number of bowel movements, beneficial effects on symptoms of constipation and a decrease in abdominal pain.

2.5.16. Antidiabetic

Calcinaro *et al.* (2005) found that oral administration of *Lactobacillus case*i induced interleukin-10 production and prevented spontaneous autoimmune diabetes in non obese diabetic mouse. Yadav *et al.* (2008) from his research findings concluded that probiotic dahi (*L. acidophilus* and *L. casei*) supplemented diet significantly delayed the onset of glucose intolerance, hyperglycaemia, hyperinsulinemia, dyslipidemia and oxidative stress in high fructose induced diabetic rats indicating a lower risk of diabetes and complications.

2.5.17. Sex Hormones

A high-fat, low-fibre diet, causes overgrowth of bacteria in the gut microflora that have the ability to convert bile acids into sex hormones, which are then absorbed

through the gut wall and into the blood stream (Hill *et al.,* 1971). The intestinal microflora play a key role in circulating estrogens in a woman's body by deconjugating bound estrogens that appear in the bile, thereby permitting the free hormones to be reabsorbed by the intestine, back into the woman's body, causing elevated hormone levels (Dupont and Page, 1985). The same effects occur in men, raising testosterone levels. Problems from excess sex hormones include: precocious puberty, fibrocystic breast disease, PMS, uterine fibroids, prostate enlargement, and breast, uterine, and prostate cancer (Peter *et al*., 1996). By changing the microflora with a low-fat, high-fibre diet and/or probiotics and prebiotics, more estrogen is excreted in the faeces, resulting in less estrogen in the body and sex-hormone related problems are prevented and improved (Gittleman, 2004).

A high-fibre, plant-based diet promotes the growth of equol, a phytoestrogen produced from the soy isoflavone daidzein by gut microflora (Lampe *et al.,* 1999). Probiotic and prebiotics would be expected to have the same effects on the microflora. Consumption of *Lactobacilli* and *Bifidobacteria* has been shown to significantly increase β-glucosidase activity in humans (Marteau *et al.*, 1990), an activity necessary to convert the isoflavone glycoside to an aglycone, and specific strains of *Bifidobacteria* have been shown to increase equol synthesis *in vitro* (Tsangalis *et al.*, 2003). Equol is a weak estrogen, it decreases the adverse effects of stronger estrogens made by a woman's body, and later in life it may provide estrogen-like benefits, such as a reduced risk for osteoporosis (Fujioka *et al.*, 2004).

2.5.18. Nutritional benefits

Probiotic bacteria break down hydrocarbons which mean the food is being split into its most basic elements. This allows almost total absorption through the digestive system. In this way probiotics dramatically increase overall nutrition and enhance rapid cellular growth and development. Probiotics also produce many important enzymes and increase the availability of vitamins and nutrients, especially vitamin B, vitamin K, lactase, fatty acids and calcium (Khetarpaul, 2005).

Many enzymes in the body require B complex vitamins (as *coenzymes*) to function. *Bifidobacteria* are able to produce some of these vitamins including B_1, B_6, and B_{12} as well as folic acid and several amino acids (Deguchi and Morishita, 1985). Acidophilus bacteria can also inhibit some of the bacteria responsible for decomposing vitamin B_1 (Honma and Ohtani, 1987).

Probiotics are responsible for several activities in the gut, including: manufacture of B vitamins including biotin, niacin, folic acid and pyridoxine (Chaitow and Trenev, 1990).

Rani and Khetarpaul (1998) reported that probiotic fermentation of indigenous food mixture RSMT containing rice, defatted soyflour, skimmed milk powder and tomato pulp in 2:1:1:1 proportion (w/w) using *L. casei* and *L. plantarum* showed a decrease of pH, increase in acidity and enhancement of the digestabilities of starch and protein. A significant ($P<0.01$) negative correlation was obtained between the contents of antinutrients.

Sindhu and Khetarpaul. (2001) developed BCGT food mixture which contained barley flour, milk coprecipitate, sprouted green gram paste and tomato pulp and reported that the fermentation drastically reduced the contents of phytic acid, polyphenols and trypsin inhibitor activity while significantly improving the *in vitro* digestibility of starch and protein and showed a good nutrient profile with crude protein content ranging from 20.87 to 21.81 per cent.

As a result of bacterial proteolysis, yoghurt has higher levels of free amino acids and improved protein digestibility and mineral bioavailabitly whereas levels of antinutrients are reduced in probiotic fermented foods (Khetarpaul, 2005).

2.6. PREBIOTICS AND SYNBIOTICS

Prebiotics are often referred to as cofactors of probiotics which can be defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health (Gibson and Roberfroid, 1995).

For a food ingredient to be classified as a prebiotic, it must 1) neither be hydrolyzed nor absorbed in the upper part of the gastrointestinal tract; 2) be a selective substrate for one or a limited number of potentially beneficial commensal bacteria in the colon, thus stimulating the bacteria to grow, become metabolically activated, or both; and 3) be able as a consequence to alter the colonic microflora towards a more healthier composition (Ziemer and Gibson, 1998).

A more recent definition stated that "a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host wellbeing and health" (Gibson *et al.,* 2005).

Prebiotic compounds are actually substances with a dietary fibre-like action which lower the intestinal pH because of the fermentation and hence lead to a decreased activity of the enzyme 7α-hydroxylase responsible for the formation of secondary bile acids which are suspected to be cytotoxic. Besides, prebiotics can be used by the intestinal flora as carbon source thus increasing the bacterial mass which is important because nitrogen and sulphur residues can also be used under these conditions for increasing the bacterial mass so that they cannot be converted into compounds with an irritant action such as H_2S, indoles and ammonia formed abundantly if a diet is rich in protein and low in dietary fibre (Crittenden *et al.*, 2005).

Compounds which are either partially degraded or not degraded by the host and are prefrentially utilised by *Bifidobacteria* as a carbon and energy source are referred to

as ‘bifidogenic factors’ and some of the bifidogenic factors with commercial significance include fructo oligosaccharide, lactose derivatives such as lactulose, lactitiol, galacto oligosaccharides and soybean oligosaccharide (O’sullivan, 1996). This role is also played by fermentable carbohydrates, which are not digested or poorly digested in small intestine and stimulate, prefrentially, the growth of bifidobactreia and some other Gram positive bacteria , belonging to the probiotic bacteria administrated to humans (Dimer and Gibson, 1998).

Food grade oligosaccharides are mixtures of saccharides with different degree of polymerisation (Crittenden and Playne, 1996). The majority of prebiotic oligosaccharides are produced on the industrial scale and are widely available in the market. Many patents concerning prebiotic oligosaccharides have been claimed and this field is continously increasing (Crittenden and Playne, 1996).

Eight different kinds of oligosaccharides have been licensed as Food for Specific Healthe Use (FOSHU) by the Ministry of Health and Welfare, Japan, including fructo-oligosaccharide, galacto-oligosaccharide, lacto- sucrose, xylo-oligosaccaride, soy bean–oligosaccharide, raffinose and isomalto oligosaccharide (Tanaka and Matsumotto, 1998).

Prebiotic oligosaccharides can be produced in different ways :by extraction from natural sources, microbiological synthesis or enzymatic synthesis, and enzymatic hydrolysis of polysaccharides (Sako *et al* ., 1999 and Gulewicz *et al*., 2003).

The basic assumption of the prebiotic is that, these indigested ingredients reach the colon and can be utilised by the intestinal flora to stimulate those bacteria (specifically *Bifidobacteria*) that are naturally part of the ecosystem in the colon (Gibson and Roberfroid, 1995 and Rastall, 2005). During the period of consumption of the prebiotic, the numbers of specific bacteria have been shown to increase by upto 100 fold, but similar to the situation with probiotics, when the consumption of the prebiotic stops, the gut bacteria numbers quickly return to their original values (Farnworth, 2001).

In vivo studies by Rowland and Tanaka (1993) and Ito *et al.* (1993) have shown that, consumption of galacto oligosaccharides resulted in the numerical predominance of *Bifidobacteria* and *Lactobacillus* in faeces. Similar results were obtained by Gibson *et al.* (1995) and Buddington *et al*. (1996) in adults who consumed fructo oligosaccharides. A daily intake of 2.5g is enough to exhibit the bifidogenic effects in healthy individuals (Sako *et al.,* 1999). Prebiotic doses higher than 20 g/day might induce some side effects such as increased flatulence or abdominal pain but prebiotics appear to have few side effects at higher doses and as existing food components (Tuohy *et al*., 2003).

Djouzi and Andrieux (1997) studied the effects of three oligosaccharides on metablosim of intestinal microflora in germ-free rats inoculated with human faecal flora and reported that fructo oligosaccharides and galacto oligosaccarides increased the *Bifidobacteria* number by 2 log cycles.

Inulin is extracted from chicory roots with hot water. Partial hydrolysis of this extract yields fructo-oligosaccharides, sometimes referred to as fructans (Roberfroid *et al*., 1998). These fructans are considered bifidogenic and increase growth of *Bifidobacterium* species in the intestinal tract, primarily in the large intestine. Inulin-type fructo-oligosaccharides have been the ones most investigated as prebiotics. Much of the focus has been on their ability to enhance growth of *Bifidobacterium* species. Fermentation, of these soluble fibres in the large intestine results in the production of short-chain fatty acids (Floch and Moussa, 1998). These fatty acids are important to the host in lipid metabolism.

Combined mixtures of probiotics and prebiotics are often used because their synergic effects conferred on to food products and for this reason, such mixtures are called synbiotics which are defined as mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract of the host (Anderson *et al* ., 2000).

Appropriate use of prebiotics and optimal combinations of probiotics and prebiotics (synbiotics) could significantly improve the manifestation of atopic dermatitis in children aged two years and over (Passeron *et al*., 2006).

Recent human studies indicate that ingestion of synbiotics modulates the gut microbiota, promoting a healthier composition; it appears that synbiotics can be more efficient than either pro- or prebiotics alone in inducing this effect. Preliminary results have shown beneficial effects on biomarkers of diseases such as ulcerative colitis (UC) and colorectal cancers (Rafter *et al*., 2007).

2.7. PROBIOTIC FOODS

Probiotics are available in foods and dietary supplements like capsules, tablets, and powders, and in some other forms as well (Fuller, 1993).

To realize the health benefits, probiotic bacteria must be viable and available at a high concentration, typically 10^{6} cfu/g of the product (Shah *et al*, 1995 and Shah et al., 2000)

Probiotic foods are those foods which contain a live microbiological culture either as a result of fermentation or as an intentional addition to beneficially affect the host by improving the intestinal microbial balance (Mark, 2002).

Traditional probiotic foods are acid fermented dairy products, such as yoghurt (Bourlioux and Pochart, 1988). Most probiotic foods are fermented at least partially and the products which have received the most attention in this regard include fermented milks, such as yoghurt and buttermilk, as well as unfermented milks with cultures added (Reuter, 1990, Sanders *et al.,* 1996 and Shah, 1997), frozen desserts such as ice cream and frozen yoghurt (Christiansen *et al*., 1996**),** miso, kefir, sauerkraut, certain pickles, tofu, tempeh etc.

The first example of a probiotic food was the introduction of *L. acidophilus* to milk, which helped people who had difficulty in digesting milk to be able to tolerate milk better (Harry and Leo, 1920*)*. However, acidophilus milk has not been successful because of its unacceptable flavour which increased the popularity of yoghurt. Yoghurt cultures are probiotics if a beneficial physiological effect can be obtained by consumption of the live cultures and the benefit has been substantiated appropriately in human studies (Gilliland & Kim, 1984; Savaiano *et al.,* 1984; McDonough *et al.,* 1987; Dewit *et al.,* 1988; Lerebours *et al.,* 1989; Pochart *et al*., 1989; Marteau *et al*., 1990; Varela- Moreiras *et al.,* 1992; Rizkalla *et al*., 2000; Labayen *et al*., 2001 and Pelletier *et al.*, 2001).

Some researchers suggested (Tramer, 1973 and Vedamuthu, 1974) that *L. acidopbilus* be incorporated into yoghurt in place of *L. bulgaricus* in making yoghurt but *L. acidophilus* grows poorly in milk used for yoghurt manufacture unless it is supplemented with more readily available nutrients (Mann and Spoerry, 1974). There have been studies showing the preventive or therapeutic control of intestinal infections by both *L. acidophilus and B. bifidum* through administering milk cultured with one or both of these organisms (Payne *et al*., 1981). Majority of the probiotic yoghurts, however, contained viable counts above 10^5 cfu per g even at the end of the best before use period (Schillinger, 1999). *S. thermophilus* and most *L. bulgaricus* strains present in yoghurt have a high lactase activity and that yoghurt consumption improves lactose digestion and eliminates symptoms of lactose intolerance (Sanders *et al*., 1996 and Rizkalla *et al*., 2000).

Takano *et al.* (1985) developed a sour milk by fermenting milk with a mixture of *Lactobacillus helveticus SS jugurti and Candida utilis* and studies indicated that rats receiving sour milk had significantly fewer tumors than did the control group.

Kefir, a cultured milk beverage that tastes similar to yoghurt, but thinner is produced by kefir grains, is a stable probiotic food that can be kept for months, in which lactose has been converted to alcohol and lactic acid (Tamai *et al.*, 1996). Adding kefir

grains to milk produces a sour yoghurt-like beverage (Katz, 2003). Recent studies have found kefir grains to include populations of *Zygosaccharomyces, Candida, Leuconostoc, Lactococcus, Lactobacillus*, and *Cryptococcus*, which grow differentially in successional phases (Witthuhn *et al*., 2005). Complete kefir fermentation produces a lactose-free food that lactose-intolerant people can digest. Kefir is being investigated as a leavener for bread (Plessas *et al*., 2005), and as starter culture for cheeses (Goncu and Alpkent 2005).A source of kefir an, an insoluble polysaccharide with antibacterial properties that show potential for use in medicines (Rodrigues *et al*., 2005).

Cheddar cheese as a probiotic food is that the microorganisms be able to survive the relatively long ripening time of at least 6 months and/or that they grow in the cheese over this period (Kosikowski, 1977). A number of nonpathogenic bacteria, chiefly *Lactobacilli* (*Lactobacillus plantarum*, *L. casei*, and *L. brevis*) and pediococci (*Pediococcus pentosaceus*) proliferate in the maturing cheese (Chapman and Sharpe, 1981). *Lactobacilli* with defined proteolytic systems have been deliberately added as adjuncts to cheese milk in order to influence cheese maturation (Trepanier *et al*., 1991 and Lynch *et al.,* 1996).

Dinakar and Mistry (1994) incorporated *Bifidobacterium bifidum* into cheddar cheese as a starter adjunct which survived well in the cheese and retained a viability of approximately 2×10^7 CFU/g of cheese even after 6 months of ripening, without adversely affecting cheese flavor, texture, or appearance. This suggested that cheddar could provide a suitable environment for the maintenance of probiotic organisms at high levels over long periods. In another study by Gomes *et al*. (1995), *Bifidobacteria* were used in combination with *L. acidophilus* strain Ki as a starter in Gouda cheese manufacture and showed a significant effect on cheese flavour in the resultant product after 9 weeks of ripening, possibly due to acetic acid production by the *Bifidobacteria*.

Gardiner *et al*. (1998) conducted a study in which probiotic *L. paracasei* strains incorporated into cheddar cheese proved particularly suitable as starter adjuncts and were found to grow and proliferate to high cell numbers in cheese over 8 months of

ripening, even when added at a relatively small inoculum. Similar work was done by Stanton *et al.* (1998) in which probiotic cheddar cheeses were manufactured containing high levels of *L. paracasei* strains (10^8 cfu g^{-1} cheese) at a relatively low cost to the producer and using identical make procedures showing positive impact on cheese quality, including aroma, flavour and texture.

Cheddar cheese was found to have a greater protective effect than yoghurt upon exposure of the probiotic culture to porcine gastric juice at pH 2 (Gardiner *et al*., 1999).

Bacteria with probiotic properties could be included with cheese starters (NLAB, 2002) or can be added directly to cheese milk or to the curd before hoping (Gardiner *et al*., 1998 and Ross *et al*., 1999). Songisepp *et al*. (2004) developed an original probiotic, semisoft cheese "Pikantne" using cheese starter cultures (Probat 505) in combination with 0.04 per cent of probiotic *Lactobacillus fermentum* strain ME-3 (10^9 cfu/mL) having good sensory properties and high antimicrobial activity and antioxidative properties.

Tempeh, is a good probiotic food source (Nout and Kiers, 2004). Tempeh contain *Rhizopus Oligosporus*, and good bacteria which produce natural antibiotics that inhibits some harmful bacteria and produce vitamins like B_{12} and can also improve intestinal digestion health, as well as skin health, from atopic dermatitis, pimples and cellulites (Liem *et al*., 1977).

Miso is a traditional Japaneese fermented or probiotic form of soyabean, which is particularly rich in the isoflavone aglycones, genistein and daizein, which are believed to be cancer chemopreventatives (Takahashi *et al.*, 1995). Their fermentation process is thought to convert the isoflavone precursor's genistein and daidzein to their active anti-cancer isoflavone forms, genistein and daidzein (Gotoh *et al.,* 1998).

Kimchi has a reputation of being a healthy food and is listed in top five "World's Healthiest Foods" for being rich in vitamins, aiding digestion, and even possibly

retarding cancer growth and one serving provides up to 80% of the daily required amount of vitamin C and carotene (Leea *et.al.,* 2005). *Kimchi* is rich in vitamin A, thiamine (B1), riboflavin (B2), calcium and iron, and contains a number of lactic acid bacteria and in 2000, a novel bacteriocin-producing lactic acid bacterium (strain MT-1077^{T}) was isolated from *Kimchi* and was named *Lactobacillus kimchi* (Yoon *et al,* 2000).

Sauerkraut is shredded, salted, and fermented white cabbage also known as a probiotic from which a strain SB900 of probiotic nature was isolated from sauerkraut fermentation, and identified as *Streptococcus lactis* which produced 1.73 per cent lactic acid and resistant to low pH (FAO, 1998). A probiotic cabbage juice by lactic acid bacteria (*Lactobacillus plantarum C3*, *Lactobacillus casei A4*, and *Lactobacillus delbrueckii D7*) was made inoculating a 24-h-old lactic culture and incubated at 30 °C and after 4 weeks of cold storage at 4 °C, the viable cell counts of *L. plantarum* and *L. delbrueckii* were still 4.1×10^7 and 4.5×10^5 mL^{-1}, respectively which could serve as a healthy beverage for vegetarians and lactose-allergic consumers (Yoon *et al*, 2006).

Certain properties relevant to probiotic action, like. resistance to acid, bile tolerance, adhesive properties, antibacterial activity, and antibiotic susceptibility were identified in *Lactobacilli* isolated from four kinds of Thai traditional fermented foods namely fermented pork, fermented fish, fermented tea leaves, and pickled garlic (Klayraung *et al.,* 2008). Dry sausages are non heated meat products and the idea of inoculating lactobacillus into this was introduced with the aim to reduce the ripening times as well as ensuring the quality and aroma of these sausages, which are said to be suitable carriers of probiotics into human gastrointestinal tract (Tyopponen *et al*., 2003).

The incorporation of *Bifidobacterium bifidum* and *L. acidophilus* in yoghurt to enhance its dietetic properties and its utilisation for the manufacture of probiotic ice cream is suggested (Holocomb and Frank, 1991; Samona and Robinson, 1994). Hekmat and McMohan (1992) developed a probiotic ice cream by fermenting a standard ice cream mix with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* cultures and

then freezing the mix in a batch freezer, showed best acceptance at pH 5.5, contained high levels of viable organisms (1.5×10^8 to 5×10^8 cfu/ml), even after 17 week of frozen storage and could be used as a good source for delivering these probiotic bacteria to the consumers. Similarly Alamprese *et al.* (2005) studied the effects of *Lactobacillus rhamnosus GG* (LGG) added to ice cream mixes in a quantity of 10^8 cfu/g and the results showed that it did not change the overrun, firmness or melting behaviour of the finished product and no count decay of LGG cells was observed in ice cream stored for up to 1 year.

Nebesny *et al*. (2007) made dark chocolate masses and chocolates supplemented with viable cells of two bacterial strains *Lactobacillus caseii* and *Lactobacillus paracasei* with potential probiotic properties, which were lyophilized in milk and total number of live bacteria in the lyophilizate was 7.9×10^9 cfu/g. The number of live *L. casei* and *L. paracasei* cells in the examined batches of chocolate were very high and approached 10^6–10^7 cfu/g after 12 months of keeping at 4 and 18 °C. In another study O'Toole (2008) studied foods that comprise a base and probiotic bacteria, particularly lactic acid forming cultures, encapsulated in chocolate or cocoa butter and is was found that after drying the weight ratio of food base to chocolate or cocoa butter encapsulated probiotic bacteria was between approximately 100:1 and approximately 100:400. He also stated that the pieces of the coated food base can be admixed with pieces of uncoated dried food base of the same or different composition to provide desired levels of probiotic fortification.

Chocolate mousse was shown to be an excellent vehicle for the delivery of *L. paracasei* and storage trials showed that the viability of the probiotic was retained over 28 days, but the growth of yeasts and moulds might limit the shelf-life of the product (Lina *et al*., 2007). Patel *et al*. (2008) developed a probiotic chocolate mousse supplementing with *L. paracasei* and in the study, viability of *L. paracasei* increased from 3.9×10^7 to 1.6×10^9 cfu /g in the probiotic chocolate mousse during 21 days of storage at refrigerator temperature.

Sindhu and Khetarpaul, (2001) developed a BCGT (Barley flour, milk coprecipitate, sprouted green gram and tomato pulp food mixture containing barley flour, milk coprecipitate, sprouted green gram paste and tomato pulp (2:1:1:1, w/w), autoclaved (1.5kg/cm2, 15min., 121°C), cooled and fermented with two per cent liquid *L.acidophilus* culture (containing 10^6 cells/ml) and fermented. The lyophilized and rehydrated food mixtures were found to be organoleptically acceptable to human palate and maintained adequate cell viability.

A probiotic fermented food mixture develped by fermentation of an unautoclaved slurry of pearl millet flour, chick pea flour, skim milk powder and and fresh tomato pulp(2:1:1:1 w/w) with *Lactobacillus acidophilus* (10^5 cells/ml) at 37° C for 24 h showed good acceptability (Rani and Khetarpaul, 1999).

A probiotic tomato juice developed by Yoon *et al*. (2004) with *Lactobacillus acidophilus* (*LA39*) having viable cell counts 10^8 /ml after fermentation for 72 h at 30° C could be served as a health beverage for vegetarians and the consumers who are allergic to dairy products. Angelov *et al.* (2006) developed an oat based probiotic drink inoculating *L plantarum* (B28) with a shelf life of 21 days under refrigerated storage.

Dalev *et al*. (2006) developed a new probiotic beverage based on cheese whey and soy with good sensory properties, and a high cell number of the probiotic bacteria (about 10^9cells/ml).

A probiotic dairy beverage consisting of *Bifidobacterium bifidum* as starter together with 15–25 per cent tomato juice, carrot concentrate or pureed pumpkin, strawberries, black mulberries or red grapes were prepared and contained levels of vitamin C, minerals and *Bifidobacterium* (approx. 10^7 cfu/g) sufficient to provide health benefits. Sensory analysis revealed that the beverages had acceptable flavour, with scores higher than those achieved by the control (Salem *et al*., 2006).

Probiotic food constitutes a sizeable part of the functional food market (Stanton *et al.,* 2001), and continues to grow at an exponential rate, with the potential for market growth estimated at a staggering US$ 120 million per month (Anon, 2001). After many years of popularity in the Japanese and European markets, manufacturers of these products are venturing into new markets, including the Arabian Gulf region, as evidenced by the variety of probiotic food products now available in supermarkets and health food stores (Senok *et al.,* 2005).

The global market for probiotic ingredients, supplements and foods was worth $14.9 billion in 2007, $15.9 billion in 2008 and reach $19.6 billion in 2013, a compound annual growth rate of 4.3%. Food applications for probiotics are found mostly with yoghurts, kefir and cultured drinks representing the major categories of probiotic foods. Emerging food applications include probiotic cheese, nutrition bars, breakfast cereal, and infant formula (BCC, 2008).

Some Commercially available probiotic foods in the Indian market

World's number one probiotics drink 'Yakult' was launched in India and this was the only probiotic drink that contains more than 6.5 billion beneficial bacteria (*Lactobacillus casei* strain), priced Rs 10 per bottle. The Yakult bacteria reach the intestines alive, to impart immense health benefits and daily consumption of 'Yakult' boosts immunity, aids digestion and prevents infections (Anon, 2007a).

India's best known dairy brand, Amul, recently have started selling sugar free probiotic diabetic delight in different flavours containing 50 percent less fat and half of the calorie than normal ice cream. Further, it has been supplemented with probiotic cultures for health improvement (Das, 2007) .This ice cream won one of the world's most prestigious awards - The International Dairy Federation Marketing Award 2007 in the nutri-marketing category (Anon, 2007b)

Nestle's 'Nesvita' is the India's first probiotic dahi containing a unique strain from *Lactobacillus acidophilus* family whose unique action in the intestine delivers

many positive benefits which lead to a healthy digestive system . This dahi is 98 percent fat free, making it the ideal food choice for a fit and healthy lifestyle (Anon, 2008).

Mother Dairy's 'B-Active Plus probiotic dahi' contains billions of friendly BB-12 bacteria which has higher survival rate in stomach resulting in better digestion and absorption of nutrients. It also contain prebiotic fibre that stimulate the activity of probiotic bacteria resulting in improved digestive health (Jacob, 2008).

2.8. SAFETY OF PROBIOTICS

According to Aguirre and Collins (1993), no pathogenic or virulence properties have been found for *Lactobacilli, Bifidobacteria* or *Lactococci*. The risk of infection by these genera is in the "negligible" range, taking into account that exposure to them is universal and persistent, not only through probiotic products but also as common colonizers of the human body (the digestive tract and oral and vaginal cavities).

The factors that must be addressed in the evaluation of safety of probiotics include pathogenicity, infectivity, and virulence factors comprising toxicity, metabolic activity, and the intrinsic properties of the microbes. Donohue and Salminen (1996) provided some methods for assessing the safety of lactic acid bacteria through *in vitro* studies, animal studies, and human clinical studies and indicated that some current probiotic strains are reported to fulfill the required safety standards. Salminen and Marteau (1997) also proposed studies on intrinsic properties, pharmacokinetics, and interactions between the host and probiotics as means to assess the safety of probiotics.

The accuracy of identification to the strain level is a critical step in the assessment of safety. Although an uncommon cause of infection in humans, a few cases of sepsis have been reported by Munoz *et al*. (2005), with the administration of the probiotic *S. cerevisiae* (boulardii). The lack of pathogenecity of *L. acidophilus* and *Bifidobacteria* extends across all age groups including pre term infants and pregnant women (Lin *et al*., 2005 and Saavedra *et al*., 2004).

Bacteria such as *Lactobacillus*, *Leuconostoc*, and *Pediococcus* species have been used extensively in food processing throughout human history, and ingestion of foods containing live bacteria, dead bacteria, and metabolites of these microorganisms has taken place for a long time (Mayra-Makein and Bigret, 1993).

Cases of infection due to *Lactobacilli* and *Bifidobacterium* are extremely rare and are estimated to represent 0.05 -0.4% of cases of infective endocarditis and bacteraemia (Saxelin *et al.*, 1996).

Naidu *et al.* (1999) opined that *Lactobacilli* have a long history of use as probiotics without established risk to humans and this remains the best proof of their safety.

The two clinical studies conducted by Wolf *et al*. (1998) and Cunnigham *et al*. (2000) to assess the safety of probiotics in small groups of specific immuno compromised patients, (eg: patients with HIV infection) support the safety of probiotics consumed by these groups.

In a safety study, four probiotic strains were fed to BALB/C mice for 4 weeks at three doses (2.5×10^{9}, 5×10^{9} and 2.5×10^{12} cfu/Kg/day) and reported to be safe for human consumption (Shu *et al.,* 1999).

Zhou *et al.* (2000) assessed the acute oral toxicity (by measuring their effect on general health status, feed intake and intestinal mucosal morphology) of mice fed with four probiotic strains on a high dose (10^{11} /mouse/day) and recovered no viable bacteria from blood and tissue samples and hence recommended an acceptable daily intake (ADI) value of 35 g of pure dry bacteria per day for a 70 Kg person.

An epidemiological study of systematically collected *Lactobacilli* bacteremia case reports has shown that there is no increased incidence or frequency of bacteremia with increased usage of probiotic *Lactobacilli* (Salminen *et al*., 2001).

It is virtually impossible to propose a risk of death because of the common association of infections involving lactobacilli with fatal underlying conditions or the presence of polymicrobial infections (Borriello *et al*., 2003).

According to Senok (2009) wide variety of probiotic products are available and development of guidelines for labelling of these probiotic products and use of structure/ function statements and health claims should be addressed.

Ideally, products labelled as probiotics would conform to the guidelines established by a working group of the FAO (UNFAO/WHO 2002). The requirement in the United States is that products be labelled in a truthful and not misleading fashion; this requirement applies to content as well as claims of functionality.

Recommendations given for safety of probiotics in US include the following: (1) Establish a standard of identity for the term "probiotic" based on the FAO definition, (2) Regulate probiotics based on their intended use, but expand regulatory conceptualization of health benefit claims, (3) Adopt the use of third party verification of label claims., (4) Use probiotics selectively in clinical conditions, (5) Consider multiple factors when evaluating probiotics, (6) Focus research on the important role of human native microbiota in health., (7) Use a science-based assessment of the benefits and risks of genetically engineered probiotic microbes and (8) Provide better information to consumers (Cast, 2007).

There is a need for standardisation in order to sift the genuine products from the artificial ones. Considering this, the Indian Council of Medical Research (ICMR), has recently set up a committee to formulate guidelines for the probiotic foods and once the guidelines are out, the products claiming to be probiotic should go through stringent

quality checks and disclosure on the labels so that the consumers can make more informed choices (Hemalatha and Rao, 2009).

Materials and Methods

3. MATERIALS AND METHODS

The methods followed and the materials used in the standardisation of probiotic fermented food mixtures and evaluation of nutritive value, organoleptic qualities and shelf life of the products are given under the following heads

3.1. Collection of raw ingredients

3.2. Enumeration of *Lactobacillus acidophilus* in the medium

3.3. Analysing the probiotic characteristics of *L. acidophilus*

3.3.1. Effect of pH on the survival of *L. acidophilus* MTCC 447

3.3.2. Effect of bile salts on the survival of *L. acidophilus* MTCC 447

3.3.3. Antibacterial activity of *L. acidophilus* MTCC 447

3.4. Standardising the proportion of ingredients in the food mixtures

3.4.1. Initial standardization of the combination of ingredients in the food mixtures

3.4.2. Acceptability of the food combinations

3.4.2.1. Selection of judges for acceptability studies

3.4.2.2. Preparation of score card

3.4.2.3. Organoleptic evaluation

3.4.2.4. Selection of the most acceptable variation in each treatment

3.5. Optimisation of variables for the fermentation of the food mixtures with *L.acidophilus*

3.5.1. Optimisation of substrate concentration

3.5.2. Optimisation of pH

3.5.3. Optimisation of temperature

3.5.4. Optimisation of time of incubation

3.5.5. Optimisation of inoculum concentration

3.6. Development of fermented food mixtures

3.6.1. Autoclaved and fermented food mixtures

3.1. COLLECTION OF RAW INGREDIENTS

Raw banana (Nendran *Musa* AAB) purchased from the local market was peeled, washed, sliced and dried. The dried chips then powdered to a flour of 40 mesh size. This flour was used as a source of starch in all food mixtures.

Defatted soya flour and green gram flour purchased from the local market and used as a source of protein in the food mixtures. Mango, tomato and papaya were purchased from the local market.

Majority of the probiotics recommended are bacteria with the species of *Lactobacillus* being the most common (Fuller, 1992). Some examples include *L. acidophilus, L. plantarum, L. casei, Streptococcus lactis* etc. In the present study, *L. acidophilus* was used as the probiotic culture for the fermentation of food mixtures.

Pure cultures of *L. acidophilus* MTCC 447 used was obtained from Institute of Microbial Technology (IMTECH), Chandigarh.

3.2. ENUMERATION OF *L.ACIDOPHILUS* IN THE MEDIUM

To find the total number of viable organisms in a fixed quantity of MRS broth, MRS broth was prepared according to the manufacturers instructions. Hundred millilitres of MRS broth was prepared and sterilized at 121^{o}C for 15 minutes. After cooling, the same was inoculated with a loopful of *Lactobacillus acidophilus.* Inoculated flasks in duplicates were incubated at 37 ^{o}C for 24 hours.

After 24 hours, 80, 100, 200 and 500 µl of the sample was serially diluted in sterile distilled water and dilutions were plated on MRS agar by spread plate method. The plates were incubated at 37 ^{o}C for 24 h. The number of colonies were counted and recorded as x 10^{6}cfu/ml.

3.3. ANALYSING THE PROBIOTIC CHARACTERISTICS OF *L. ACIDOPHILUS* MTCC 447

Probiotic organisms for use in foods, should be capable of surviving in the digestive tract and should also have the capacity to proliferate in the gut (Playne, 1994). Factors such as gastric juice with its antibacterial effect, low pH and bile salt concentration are factors which inhibit the growth or kill many bacteria in the gut (Marteau *et al.*, 1993 and Simon and Gorbach, 1987). Hence, the following experiments were undertaken to study certain probiotic characteristics of *L. acidophilus* (MTCC 447) with the final aim to use it as a probiotic culture for preparation of fermented food mixtures.

3.3.1. Effect of pH on the survival of *L.acidophilus* MTCC 447

The survival of any organism in the stomach should be pH -HCl dependent (Giannella *et al*., 1972). *L. acidophilus* (MTCC 447) was tested for its activity to resist the action of gastric acidity

MRS broth was prepared and 10 ml was dispensed into each conical flasks. Medium was adjusted to pH 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 using pH meter and inoculated with 0.1ml of 24 hour old culture and subsequently incubated the flasks for 24 h at 37 ˚C.

After incubation, 100 µl of the sample from each flask was serially diluted in sterile peptone water and plated on MRS medium by spread plate method. The plates were incubated at 37˚ C for 24 h. The number of colonies were counted and expressed as x 10^9cfu/ml

3.3.2. Effect of bile salts on the survival of *L. acidophilus* MTCC 447

Bile acid tolerance is an essential criteria for a probiotic organism for colonization in the colon (Huis and Havenaar *et al*., 1992).MRS broth was prepared and 10ml of the broth was distributed to each conical flask. The bile acid concentration in human intestine varies in different regions (jejunum -4 percent bile, ileum-2 percent bile, large intestine-1.5 percent bile) (Chou and Weimer, 1999). Hence bile salt was adjusted to 1 to 4 percent levels in the media. A control was maintained without bile salt. The medium was sterilized at 121^{o}C for 15 minutes.

After cooling 0.1ml of 24 h grown inoculum of *L. acidophilus* was added to the flasks and kept for incubation at 37 ^{o}C for three hour. After each hour of incubation 100µl of the sample was serially diluted in sterile peptone water and sensible dilution were plated on the MRS medium by spread plate method and incubated at 37^{o}C under anaerobic conditions for 48 h. The number of colonies were counted and recorded as x 10^{5}cfu/ml.

3.3.3. Antibacterial activity of *L.acidophilus* MTCC 447

Probiotic organisms exhibit antagonistic action towards enteropathogens such as *Escherichia coli, Shigella, Salmonella, Staphylococcus, Bacillus, Proteus* etc (Khedkar *et al.*, 1998) .Hence in this experiment an attempt was made to study the antibacterial activity of *L. acidophilus* MTCC 447 for use in the preparation of fermented food mixtures to be consumed by humans. The enteropathogens tested were strains of *E.coli* MTCC 40, *Salmonella enteritidis* MTCC 3219, *Bacillus cereus* MTCC 430, *Staphylococcus aureus* MTCC 430 and *Shigella flexineri* MTCC 1457. All these cultures were obtained from IMTECH, subcultured in nutrient agar and maintained at 4 to 8 ˚C.

The mode of inhibition of *L. acidophilus* was determined by agar well assay (Singh and Sharma, 1999). Saline suspensions (0.85%) of the pathogens were made using sterile cotton swab; lawn culture of the pathogen was made in nutrient agar in sterile plates by streaking the entire agar surface. Plates were allowed to set and dry. Wells of 5mm diameter were cut with sterile well borer in each plate. MRS broth of 25ml was prepared and distributed to each conical flask after adjusting the pH at 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0. The medium was sterilized at 121˚C for 15 minutes. After cooling, 0.1ml of 24 h grown inoculum was added to this and was incubated at 37˚ C for 24 h.

From this *L. acidophilus* cultures, 200 µl (116×10^6 cfu/ml) were used to fill each well in the agar plate. The plates were then incubated at 37˚C for 24 h. Diameter of the clear zone around wells was measured in mm.

3.4. STANDARDISING THE PROPORTION OF INGREDIENTS IN THE FOOD MIXTURES

3.4.1. Initial standardisation of the combination of ingredients in the food mixtures

Fourteen food mixtures using banana flour, defatted soya flour, green gram flour, ripe mango, papaya and tomato pulp each with four variations (total 56 combinations) were prepared and the proportion of ingredients used is given in Table 1.

Table 1. Proportion of ingredients in the food mixtures

Food Mixtures (Treatments)	Combination of ingredients	Variation (per cent)					
		B	DS	GG	M	P	T
T_1	B+DS+M	50	30	-	20	-	-
		50	25	-	25	-	-
		60	20	-	20	-	-
		70	20	-	10	-	-
T_2	B+DS+P	50	30	-	-	20	-
		50	25	-	-	25	-
		60	20	-	-	20	-
		70	20	-	-	10	-
T_3	B+DS+T	50	30	-	-	-	20
		50	25	-	-	-	25
		60	20	-	-	-	20
		70	20	-	-	-	10
T_4	B+GG+M	50	-	30	20	-	-
		50	-	25	25	-	-
		60	-	20	20	-	-
		70	-	20	10	-	-
T_5	B+GG+P	50	-	30	-	20	-
		50	-	25	-	25	-
		60	-	20	-	20	-
		70	-	20	-	10	-
T_6	B+GG+T	50	-	30	-	-	20
		50	-	25	-	-	25
		60	-	20	-	-	20
		70	-	20	-	-	10
T_7	B+DS+M+P	50	30	-	10	10	-
		50	25	-	12.5	12.5	-
		60	20	-	10	10	-
		70	20	-	5	5	-
T_8	B+DS+M+T	50	30	-	10	-	10
		50	25	-	12.5	-	12.5
		60	20	-	10	-	10
		70	20	-	5	-	5
T_9	B+DS+P+T	50	30	-	-	10	10
		50	25	-	-	12.5	12.5
		60	20	-	-	10	10
		70	20	-	-	5	5

T_{10}	B+GG+M+P	50	-	30	10	10	-
		50	-	25	12.5	12.5	-
		60	-	20	10	10	-
		70	-	20	5	5	-
T_{11}	B+GG+M+T	50	-	30	10	-	10
		50	-	25	12.5	-	12.5
		60	-	20	10	-	10
		70	-	20	5	-	5
T_{12}	B+GG+P+T	50	-	30	-	10	10
		50	-	25	-	12.5	12.5
		60	-	20	-	10	10
		70	-	20	-	5	5 -
T_{13}	B+DS+M+P+T	50	30	-	6.67	6.67	6.67
		50	25	-	8.34	8.34	8.34
		60	20	-	6.67	6.67	6.67
		70	20	-	3.34	3.34	3.34
T_{14}	B+GG+M+P+T	50	-	30	6.67	6.67	6.67
		50	-	25	8.34	8.34	8.34
		60	-	20	6.67	6.67	6.67
		70	-	20	3.34	3.34	3.34

(B-Banana flour, DS-Defatted soya flour, GG-Green gram flour, M-Mango pulp, P-Papaya pulp, T-Tomato pulp)

Banana flour and fruit pulps were prepared. The developed mixtures in their appropriate proportion of ingredients (100g) were mixed with 600ml of distilled water, stirred to obtain a uniform slurry and autoclaved at 1.5 kg / cm^2 for 15 minutes. It was then cooled and inoculated with 100 µl liquid cultures of *L. acidophilus* (24 h old culture). The culture used for inoculation contained $107x10^6$ cfu/ml of broth. Fermentation was carried out at 37ºC for 24 h. After fermentation, the slurry of each food mixture was freeze dried at the central laboratory of Veterinary College, Mannuthy (Plate 1). The freeze dried fermented food blends were subjected to organoleptic evaluation.

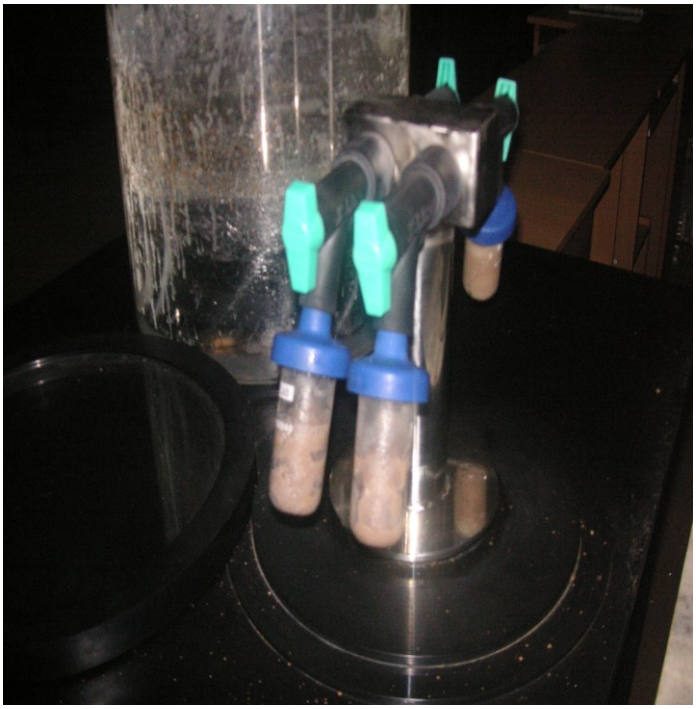


Plate 1. Freeze drying of food mixtures

3.4.2. Acceptability of the food combinations

3.4.2.1. Selection of judges for acceptability studies

A series of acceptability trials were carried out using simple triangle test at the laboratory level and selected a panel of ten judges between the age group of 18-35 years as suggested by Jellinek (1985)

3.4.2.2. Preparation of score card

Score cards were prepared for the evaluation of the food mixtures and this is given in Appendix I and II.

3.4.2.3. Organoleptic evaluation

Sensory evaluation of the developed food mixtures in powder form were carried out in the morning using score cards based on a five point hedonic scale (Appendix I) by a panel of 10 selected judges. The quality attributes namely appearance, colour, flavour, texture, taste, and overall acceptability were evaluated.

3.4.2.4. Selection of the most acceptable variation in each treatment

The best proportion of ingredients in each treatment was selected based on the acceptability scores by applying Kendall's coefficient of concordance

3.5. OPTIMISATION OF VARIABLES FOR THE FERMENTATION OF THE FOOD MIXTURES WITH *L.ACIDOPHILUS*

Optimisation is a process by which a numeric function is maximized or minimized, while satisfying all the constraints on the variable. Hence, using *L. acidophilus* for fermentation, total viable count in the product was maximized

while variables like substrate concentration, quantity of the inoculum, time of incubation, pH and temperature were kept at acceptable levels.

3.5.1. Optimisation of substrate concentration

From each food combination selected (14 food mixture combinations) weighed 25g, 50g and 75g and made slurry mixing with 150 ml of water in conical flasks, autoclaved at 121° C for 15 minutes. This was allowed to cool and was inoculated with 100µl (107 $\times 10^6$ cfu/ml) of 24 h old culture of *L .acidophilus*. The flasks with samples were incubated at 37° C for 24 h. After 24 h, the samples were freeze dried and were enumerated for viable counts of *L. acidophilus.*

Viable counts of *L. acidophilus* present in fermented food mixtures were enumerated using MRS medium. One gram of the mixture was weighed and transferred to a tube containing 9ml sterile distilled water (dilution 10^{-1}). This was then serially diluted upto 10^{-7}. The samples were enumerated for microbial count by pour plate method using MRS agar and the results are expressed as x 10^7 cfu/g.

3.5.2. Optimisation of pH

The best substrate concentration (with maximum viable count of *L.acidophilus*) was taken and slurries were prepared with 150 ml water and the pH was adjusted to 3.5, 4.5, 5.5 and 6.5 using citric acid (20 percent). Autoclaved at 121°C for 15 minutes, cooled and inoculated with 100µl (107 $\times 10^6$ cfu/ml) of 24 h old culture of *L.acidophilus*, incubated at 37° C for 24 h. After 24 h, the samples were freeze dried and were enumerated for viable counts of *L. acidophilus*

3.5.3. Optimisation of temperature

Each food combination with best substrate concentration was taken and slurries were prepared with 150 ml water and adjusted to the selected optimum pH. Autoclaved at 121°C for 15 minutes, cooled and inoculated with 100µl (107 $\times 10^6$ cfu/ml) of 24 h old culture of *L.acidophilus* and incubated at varying temperatures of 37° C, 41° C and 45 °C for 24 h. After 24 h, the samples were freeze dried and were enumerated for viable counts of *L. acidophilus.*

3.5.4. Optimisation of time of incubation

Each food combination with best substrate concentration was taken and slurries were prepared with 150 ml water and adjusted to the optimum pH. The slurries were autoclaved, cooled and inoculated with 100µl (107 $\times 10^6$ cfu/ml) of 24 h old culture of *L.acidophilus*, incubated at the optimum temperature for varying periods of 18 hours, 24 h and 30 h. After this, the samples were freeze dried and were enumerated for viable counts of *L. acidophilus.*

3.5.5. Optimisation of inoculum concentration

Each food combination with best substrate concentration was taken and slurries were prepared with 150 ml water and adjusted to the optimum pH. The slurries were autoclaved at 121 °C for 15 minutes, cooled and inoculated with varying inoculum concentration of 100µl (107 $\times 10^6$ cfu/ml), 200µl (116 $\times 10^6$ cfu/ml) and 300µl (119$\times 10^6$ cfu/ml) and kept for incubation at the optimum temperature for the optimum period of fermentation. After fermentation, the samples were freeze dried and were enumerated for viable counts of *L. acidophilus.*

3.6. DEVELOPMENT OF FERMENTED FOOD MIXTURES

After optimising the variables for fermentation, each treatment of food mixture was fermented under optimum conditions with two controls. The treatments and control samples are listed below and fermentations were carried out in triplicates.

3.6.1. Autoclaved and fermented food mixtures

Each developed food mixture (25 g) was mixed with 150 ml water and stirred to obtain uniform slurry. Adjusted the pH to 4.5 and autoclaved at 121° C (1.5 kg/cm^2) for 15 minutes. After cooling, this was inoculated with 300µl(119×10^6 cfu/ml) liquid culture of *L. acidophilus* (24 hour old culture) and incubated at 37° C for 24 h. After fermentation, it was freeze dried.

3.6.2. Fermented food mixtures without autoclaving

Each developed food mixture (25g) was mixed with 150ml water and stirred to obtain uniform slurry. Adjusted the pH to 4.5 and inoculated with 300µl (119×10^6 cfu/ml) liquid culture of *L.acidophilus* (24 hour old culture) and incubated at 37° C for 24 h. After fermentation, it was freeze dried.

.

3.6.3. Autocalved and unfermented food mixtures

Each developed food mixture (25 g) was mixed with 150 ml water and stirred to obtain uniform slurry. Adjusted the pH to 4.5 and autoclaved at 121° C (1.5 kg/cm^2) for 15 minutes. After cooling, it was freeze dried.

3.7. QUALITY EVALUATION OF THE FOOD MIXTURES

Quality aspects such as chemical composition, *in vitro* digestibility of starch and protein, population of *L. acidophilus* and other microbial contaminants and sensory qualities (using 9 point hedonic scale as in Appendix 11) were assessed in the food mixtures.

3.7. 1. Chemical constituents of the food mixtures

Analysis was carried out with three replications of each treatment.

3.7.1. 1.Moisture

Moisture content of the food mixtures was estimated by the method of A.O.A.C (1980).

To determine the moisture content five gram of sample was taken in a petridish and dried at 60-70°C in a hot air oven, cooled in a desiccator and weighed. The process of heating and cooling was repeated till constant weight was achieved. The moisture content of the sample was calculated from the loss in weight during drying.

3.7.1.2. Titrable Acidity

Titrable acidity in the food mixtures was estimated by the method suggested by Ranganna (1986). The extract was prepared by boiling a weighed quantity of the food mixture in distilled water. An aliquot of the extract was titrated against 0.1N sodium hydroxide (NaOH) using phenolphthalein indicator. Acidity was expressed in terms of g lactic acid /100g.

3.7.1.3. Starch

Starch content was estimated colorimetrically using anthrone reagent, as suggested by Sadasivam and Manikam (1992).

Weighed 0.5 g of the sample and extracted with 80 per cent ethanol to remove sugars. Residue was repeatedly extracted with hot 80 per cent ethanol to remove the sugars completely. The residue was dried over a water bath and added five milliliter water and 6.5 ml of 52 per cent perchloric acid and extracted in the cold for 20 minutes. Centrifuged the sample and re-extracted with fresh perchloric acid. The supernatant was pooled and made up to 100 ml. Pipetted out 0.2 ml of the supernatant and made upto one milliliter with water and added four milliliter of anthrone reagent, heated for 8 minutes, cooled and read the OD at 630 nm.

A standard graph was prepared using serial dilutions of standard glucose solution. From the graph, glucose content of the sample was obtained.

3.7.1.4. Total soluble solids

Total soluble solids of the food mixtures was recorded using a hand refractometer at room temperature and the values were expressed in degree brix (Ranganna, 1986).

3.7.1.5. Reducing and total sugars

Reducing sugar was estimated by the method given by Lane and Eyon (Ranganna, 1986). To ten gram of the sample, 100 ml distilled water was added and then clarified with neutral lead acetate. Excess lead was removed by adding potassium oxalate. The volume was then made upto 250 ml. An aliquot of this solution was titrated against a mixture of Fehlings solution A and B using methylene blue indicator. The reducing sugar was estimated as percentage.

From the clarified solution used for the estimation of reducing sugars, 50 ml was taken and boiled gently after adding citric acid and water. It was later neutralized with sodium hydroxide and the volume was made upto 250 ml. An

aliquot of this solution was titrated against Fehlings A and B. The total sugar content was expressed as percentage.

3.7.1.6. Protein

Protein was estimated by the method of A.O.A.C (1980).

The sample (0.3 g) and was digested with 6 ml conc H_2SO_4 after adding 0.4 g of $CuSO_4$ and 3.5 g K_2SO_4 in a digestion flask until the colour of sample was converted to green. After digestion it was diluted with water and 25 ml of 40 per cent NaOH was pumped. The distillate was collected in 2 per cent boric acid containing mixed indicators and then titrated with 0.2 N HCl.

3.7.1.7. β carotene

β carotene was estimated by the method of A.O.A.C (1970) using saturated n-butanol. To five gram of the sample, 50 ml of saturated butanol was added and shaken for a minute and kept overnight. Decanted the supernatant and read the colour intensity at 435 nm in a spectrophotometer. The β carotene was expressed in µg per 100 g.

3.7.1..8. Fibre

Crude fibre content was estimated by acid-alkali digestion method as suggested by Chopra and Kanwar (1978).

Two gram of sample was boiled with 200 ml of 1.25 per cent sulphuric acid for 30 minutes. It was filtered through a muslin cloth and washed with boiling water and again boiled with 200 ml of 1.25 per cent sodium hydroxide for 30 minutes. Repeated the filteration through muslin cloth and washed with sulphuric acid, water and alcohol in a sequential manner. Transferred the residue to a pre-weighed ashing dish. The residue was ignited for 30 minutes in a muffle

furnace at 250°C, cooled in a desicator and weighed. The fibre content of the sample was calculated from loss in weight on ignition.

3.7.1.9. Calcium

Calcium was estimated by titration method with EDTA as suggested by Page (1982).

Five ml of diacid extract made upto 100 ml was taken and added 100 ml water, 10 drops of hydroxylamine, 10 drops of triethanol amine and 2.5 ml of NaOH and 10 drops of calcone. Then it was titrated with EDTA till the appearance of permanent blue colour. It was expressed in mg per 100 g of the sample.

3.7.1.10. Potassium

The method suggested by Jackson (1973) was followed for the estimation of potassium using a flame photometer

One gram of the digested solution was made up to 25 ml and read directly in a flame photometer. The potassium content was expressed in mg per 100 gm of the sample.

3.7.1.11. Iron

Iron was estimated by Atomic Absorption Sectrophotometric method using the diacid extract prepared from the sample (Perkin-Elmer, 1982).

3.9.12. Thiamine

Thiamine content was estimated as suggested by Sadasivam and Manikam (1992). Five gram finely ground sample was taken into a 250 ml conical flask in duplicate.

Slowly added 100ml 0.1N sulphuric acid without shaking, and was kept overnight. After shaking vigorously, filtered through whatman No.1 filter paper and discarded the first 10-15 ml of the filtrate. Pipetted out 10ml of the extract in duplicate into 100 ml separating funnels. Pipetted out 10ml of working standard and added 3 ml of 15 % NaoH into each separating funnel immediately followed by four drops (0.2 ml) of ferricyanide solution. After shaking gently for exactly 30 seconds, added 15 ml of isobutanol rapidly from a quick delivery burette. Stoppered immediately, shook vigorously for 60 seconds and allowed the layers to separate. Drained off the bottom layer carefully and discarded it and added one spatula full of sodium sulphate directly into the separating funnel, stoppered and swirled gently to clarify the extract. The clear extract was collected from the top into a clean dry test tube and read at an excitation wave length of 365 nm and emission wave length 435 nm, excitation band pass and emission band pass of 10nm and sensitivity set at 500 V in a spectroflurometer. The thiamine content was expressed as mg per 100gm of the sample

3.7.1.13. Riboflavin

Riboflavin content was estimated as suggested by Sadasivam and Manikam (1992).

Dissolved five milligram of riboflavin standard in 100ml standard flask with five percent acetic acid. The flask was then covered with aluminum foil to prevent decomposition of riboflavin .Further diluted to give 10 ppm with five percent acetic acid. Blank was set at 5 percent acetic acid.

Weighed two gram of the sample into 250 ml conical flask and added 75 ml 0.1N H_2SO_4, autoclaved the mixtures for 30 minutes. Cooled and added 5 ml of 2.5 molar sodium acetate solutions and kept for 1 hour. Transferred to volumetric flask and made upto 100 ml. Filtered and discarded the first 10-15 ml. Ten ml of the sample solution was taken and added two ml of water and one ml of potassium permanganate (4 percent) solution, kept for two minutes and then added 1 ml of hydrogen peroxide solution and was read immediately in the spectroflurometer with an excitation wave length of 390nm, emission wave length of 520 nm, excitation band pass and emission band pass at 10 nm and with an EHT of 550 Volt. The riboflavin content was expressed as mg per 100gm of the sample

3.7.2. *in vitro* starch digestibility(IVSD)

Starch digestibility was estimated as suggested by Satterlee *et al.* (1979).One gram of the sample in 100ml water was gelatinised and boiled for one hour and filtered One ml of the gelatinised solution was taken and one ml of the enzyme solution (saliva diluted with equal quantity of water) was added. The mixture was incubated at 37 ° C for 1- 2 hours. The reaction was stopped by adding 1ml of sodium hydroxide. Later glucose was estimated by the method of Somoygi (1952)

3.7.3. *in vitro* protein digestibility(IVPD)

The method proposed by Sadasivam and Manikam (1992) was used to determine IVPD. A multi-enzyme system, consisting of a mixture of porcine pancreatic trypsin type IX, bovine pancreatic chymotrypsin type II and porcine intestinal peptidase grade III, was used. Food mixtures and distilled water were used to prepare 50 ml of an aqueous protein suspension (6.25 g protein/l) with pH adjusted to 8.0, while stirring in a water bath at 37 °C. The multi-enzyme solution

was maintained in an ice bath. Five ml aliquots of the multi-enzyme solution were added with stirring to the protein suspension at 37 °C. The rapid pH drop was recorded automatically over a 10 minutes period using a pH meter. IVPD was calculated from the equation IVPD = 210.46 − 18.10*X*, where *X* = pH after 10 minutes.

3.7.4. Microbial enumeration and viable count of *L.acidophilus* in the food mixtures during storage

The total microbial count in the food mixtures were enumerated by serial dilution and plate count method as described by Agarwal and Hasija (1986).Ten gram of powdered sample was added to 90 ml sterile water and agitated for 20 minutes. One ml of this solution was transferred to a test tube containing 9ml of sterile water to get 10^{-2} dilution and similarly 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}dilutions were also prepared.

Enumeration of total microbial count was carried out using nutrient agar media for bacteria, Potato dextrose agar media for fungus and Sabouraud's dextrose agar media for yeast. The dilution used for bacteria was 10^{-7} whereas for fungus and yeast 10^{-3} dilution were used

Viable counts of *L.acidophilus* in food mixtures were enumerated as described in 3.5.1

3.7.5. Organoleptic evaluation of the food mixtures

Judges were selected for acceptability studies as described in 3.4.2.1. Sensory evaluation of the developed food mixtures were conducted by mixing 5g of the food mixture in 100 ml of diluted buttermilk (1:4) and was done in the morning using score cards based on 9 point hedonic scale as in Appendix II (which is used recently in sensory evaluation studies of foods) by the selected

panel of 10 judges. The quality attributes namely appearance, colour, flavour, texture, taste and overall acceptability were evaluated.

3.8. SELECTION OF SIX FOOD MIXTURES WITH MAXIMUM QUALITY ATTRIBUTES

From the fourteen fermented food mixtures, six food mixtures with maximum quality attributes were selected by applying geometric mean scores.

3.9. STORAGE STUDIES OF THE SELECTED FOOD MIXTURES

The six food mixtures selected along with their controls were packed in metalised polyester polyethylene laminated pouches and were stored for a period of six months under ambient conditions. Quality evaluation of the stored food mixtures were conducted for each month as detailed under 3.7.

3.10. SELECTION OF FOOD MIXTURES WITH MAXIMUM QUALITY ATTRIBUTES

From the six fermented food mixtures stored for a period of six months, three food mixtures with maximum quality attributes after storage were selected by applying geometric mean scores.

3.11. MODIFICATIONS IN THE COMPOSITION OF FOOD MIXTURES

Even though there was a reduction in the viable count of *L. acidophilus* during storage, the desired level of 10^6 cfu/g for a probiotic food was maintained in all the food mixtures. Since the objective of the study is to develop probiotic fermented food mixtures with good shelf life and acceptability, substances which can enhance and prolong the growth and viability of *L. acidophilus* has to be selected as added ingredients in the food mixtures.

As reported by Sanders and Klaenhammer (2001), *L. acidophilus* is homofermentative microbes which can catalise large amounts of substrates to generate energy for growth. In addition to glucose, *L. acidophilus* utilizes cellobiose, galactose, lactose, maltose, sucrose and trehalose. Hence, in the present study sucrose, skimmed milk powder (as a source of lactose and galactose), wheat bran (as a source of cellobiose) and sorbitol as a sugar substitute were selected as substrate constituents in the selected three food mixtures.

3.11.1. Standardising the level of additives in the food mixtures.

3.11.1.1. Sucrose

Sucrose was added at 5, 10 and 15 per cent level in each of the selected three food mixtures before fermentation. Viable count of *L. acidophilus* was enumerated in the freeze dried samples.

3.11.1.2. Sorbitol

Sorbitol was added at 5, 10 and 15 per cent level in each of the selected three food mixtures before fermentation. Viable count of *L. acidophilus* was enumerated in the freeze dried samples

3.11.1.3. Wheat bran

Wheat bran was added at 5, 10 and 15 per cent level in each of the selected three food mixtures before fermentation. Viable count of *L. acidophilus* was enumerated in the freeze dried samples

3.11.1.4. Skimmed milk powder

Skimmed milk powder was added at 5, 10 and 15 per cent level in each of the selected three food mixtures before fermentation. Viable count of *L. acidophilus* was enumerated in the freeze dried samples

3.11.2. Storage studies of the selected modified food mixtures

The selected modified food mixtures with maximum viable counts of *L. acidophilus* along with their control (without substrate modification) were packed in metalised polyester/ polyethylene laminated pouches and were stored for a period of six months under ambient conditions. Quality evaluation of the stored food mixtures were conducted for each month as detailed in 3.7.

3.12. STATISTICAL ANALYSIS

The data was analysed by applying statistical techniques such as Kendall's coefficient of concordance, Duncans multiple range test, geometric mean scores and independent sample't' test.

Results

4. RESULTS

The results pertaining to the study entitled "Standardisation and quality evaluation of banana based probiotic fermented food mixtures" are presented in this chapter

4.1. Enumeration of *L. acidophilus* in the medium.

The total number of viable *L. acidophilus* present in MRS broth is given in Table 2.

Table 2. Total viable count of *L. acidophilus* from MRS broth

Quantity of MRS broth (µl)	x 10^6 cfu/ml
80	74
100	107
200	116
500	125

As revealed in the table the viable count increased as the quantity of broth increased. But the best inoculum was considered as 100µl since 100µl of the inoculum contained countable and isolated colonies from 10^{-4} to 10^{-7} dilutions. Thus 100µl of the inoculum was used to inoculate for further studies.

4.2. Probiotic characteristics of *L. acidophilus* MTCC 447

4.2.1. Effect of pH on the survival of *L. acidophilus*

The viable count of *L. acidophilus* at different pH is given in Table 3.

Table 3 . Survival of *L .acidophilus* at different pH

pH	×10^9cfu/ml
1.5	Nil
2.0	27
2.5	43
3.0	75
3.5	89
4.0	78
4.5	76

Table 3 continued

pH	×10 ^{9}cfu/ml
5.0	73
5.5	71
6.0	50
6.5	35
7.0	32
7.5	31
8.0	29
8.5	26
9.0	22

The values are mean of 3 independent enumerations

In the present study, viable count was observed between pH 2.0 and 9.0 and it was observed that the viable count varied with different pH (Fig 1). At pH 1.5 there was no growth and with an increase in pH, the viable count increased and reached a maximum of 89 x 10^{9}cfu/ml at pH 3.5 and then gradually decreased and minimum viable count was observed at pH 9.0 (22 ×10 ^{9}cfu/ml) (Plate 2).

4.2.2. Effect of bile salts on the survival of *L. acidophilus* MTCC 447

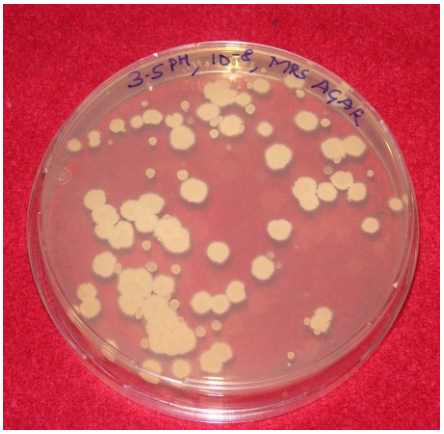
Effect of bile salts on the survival of *L. acidophilus* MTCC 447 is given in Table 4.

Table 4. Effect of bile salts on the survival of *L. acidophilus*

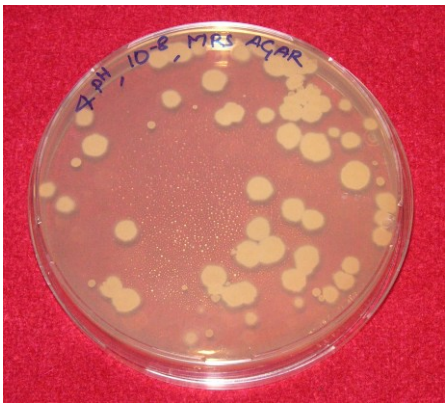
Bile Salt %	Incubation Period (h)	Population (× 10^{5} cfu/ml)	% Reduction
1	1	289	20
	2	223	40
	3	106	71
2	1	197	31
	2	115	60
	3	62	78
3	1	40	67
	2	19	85
	3	0	0
4	1	9	86
	2	0	0
	3	0	0

Values are mean of 3 independent enumerations

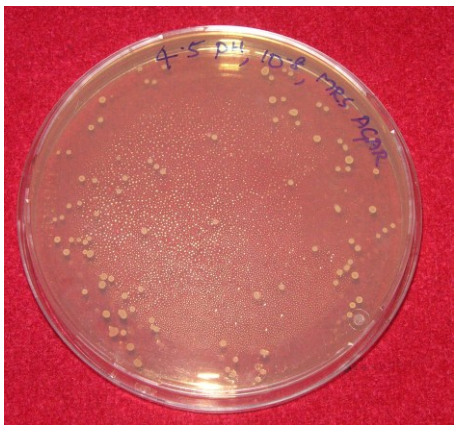
Plate 2. Viable count of *L. acidophilus* at different pH levels



Viable count of *L. acidophilus* at 3.5 pH



Viable count of *L. acidophilus* at 4 pH



Viable count of *L. acidophilus* at 4.5 pH

In all the cases, population was highest in control from which percentage reduction was worked out. As revealed, a bile salt concentration of 1-2 per cent up to three hours was tolerable by *L. acidophilus* culture and as the time of incubation increased there was an increase in the reduction of viable counts. Viable counts were observed only upto two hours of incubation for three per cent bile salt concentration, while for four per cent bile salt concentration, viable counts were observed only for one hour incubation (Fig 2)

4.2.3. Antibacterial activity of *L. acidophilus* on enteropathogens

Table 5. Antagonistic activity of *L .acidophilus* MTCC 447 at varying pH

pH / Test Organisam	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0
	Inhibition zone in mm								
E.coli	20	16	17	17	16	16	15	13	12
Bacillus cereus	18	18	14	13	13	12	11	11	10
Staphylococcus aureus	15	14	14	13	12	12	11	11	10
Salmonella enteritidis	24	24	22	19	18	18	17	16	8
Shigella flexneri	5	5	5	5	5	5	5	5	5

- Zone of inhibition including the well diameter of 5mm

As revealed in table 5, Antagonistic activity of *L.acidophilus* was highest at pH 3.0 on all selected enteropathogens.

E.coli was inhibited by *L.acidophilus* and maximum inhibition was at pH 3.0 with a zone of inhibition 20mm. As the pH increased, zone of inhibition was found to be decreasing. At pH 7.0, minimum inhibition was observed with a zone of 12mm. At pH 4.0 and 4.5, the zone of inhibition was constant at 17mm. At pH 5.0 and 5.5 the zone of inhibition was constant at 16mm. There was a gradual decrease in the zone of inhibition from pH 3.0 – 7.0 (Plate 3)

Bacillus cereus was inhibited by *L.acidophilus* in all pH from 3.0-7.0, the maximum being at pH 3.0 and 3.5(18mm). At pH 4.0, there was a sharp decrease in zone of inhibition to 14mm. From pH 4.0 there was a gradual decrease in the inhibition

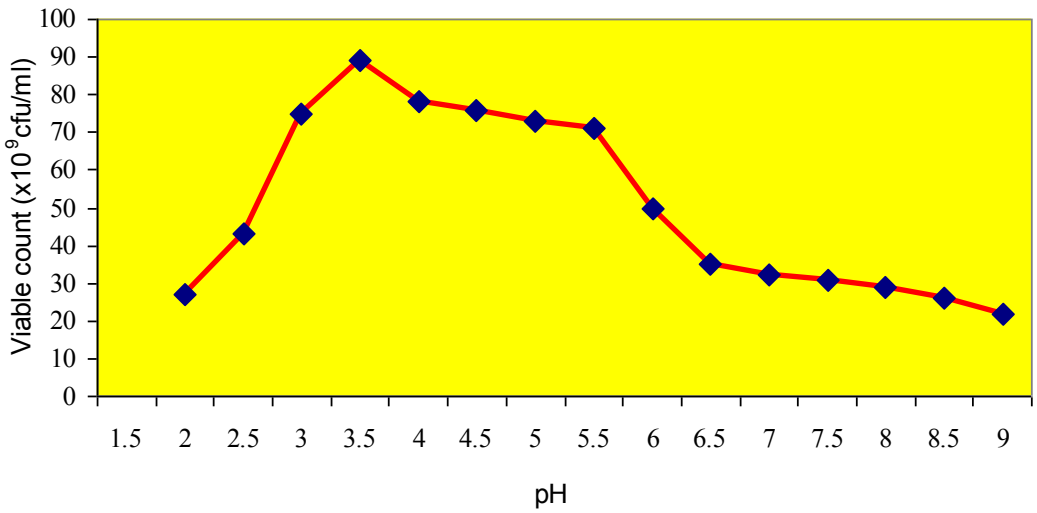


Fig .1. Survival of *L. acidophillus* at different pH

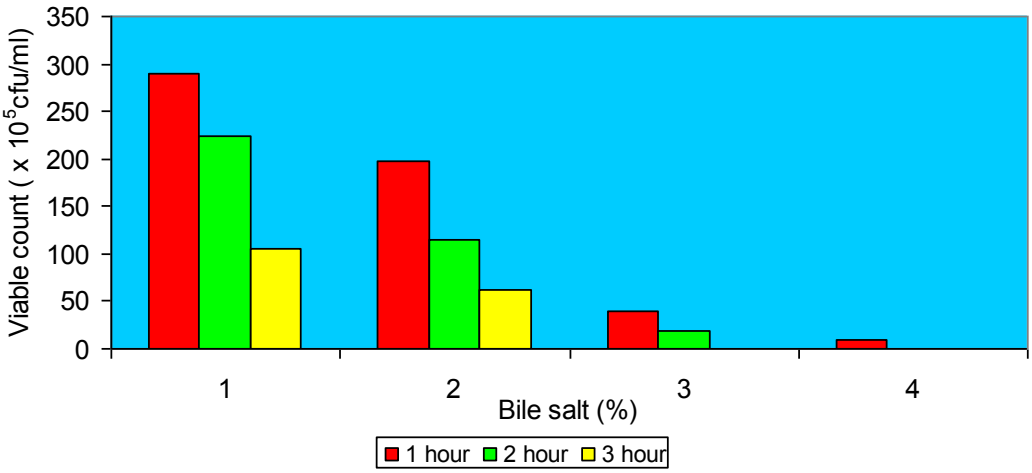


Fig. 2. Effect of bile salts on the survival of *L.acidophillus*

zone. The minimum zone of inhibition was at pH 7.0 (10mm). The zone of inhibition remained constant at pH 4.5-5.5 (13mm) and at pH 6.0-6.5 (11mm). (Plate 4).

Maximum inhibition of *Staphylococcus aureus* was observed at pH 3.0 (15mm) and the minimum was at pH 7.0 (10mm). The zone of inhibition remained constant at pH 3.5-4.0 (14mm) which decreased to 13mm at pH 4.5 and again decreased to 12mm at pH 5.0 and remained constant up to pH 5.5. The zone of inhibition was 11mm at pH 6.0 and 6.5 and again decreased to 10mm at pH 7.0. There was a gradual decrease in the zone of inhibition from pH 3.0-7.0. (Plate 5).

Among all the enteropathogens, maximum inhibition by *L.acidophilus* was on *Salmonella enteritidis* upto pH 6.5. At pH 3.0-3.5, maximum inhibition of *Salmonella enteritidis* was observed by a zone of inhibition of 24mm. As the pH increased from 4.0 to 5.0 there was a gradual reduction in the zone of inhibition from 22mm to 18mm. Upto pH 5.5 this inhibition was constant with 18mm.This was reduced to the least zone of inhibition of 8mm with pH 7.0. The zone of inhibition was 17mm at pH 6.0 and 16mm at pH 6.5 which was the highest zone of inhibition when compared to other enteropathogens at the same pH (Plate 6)

Among all the pathogens studied, *Salmonella enteritidis* was inhibited by *L. acidophilus* most effectively with an inhibition zone of 24mm at pH 3.0, followed by *E. coli* (20mm), *Bacillus cereus* (18mm) and *Staphylococcus aureus* (15mm). *L. acidophilus* was not capable of inhibiting *Shigella flexneri* at any of the pH.
The pattern of inhibition of *L. acidophilus* is depicted in Fig (3)

4.3. Standardising the proportion of ingredients in the food mixtures

Fourteen food mixtures, each with four variations were evaluated by scoring for organolpetic qualities such as appearance, colour, flavour, texture and taste by a semi trained panel of 10 judges using a five point hedonic scale. Acceptability of the combinations based on the mean score obtained are presented in the Table 6

Antagonistic activity of *L. acidophilus* on enteropathogens

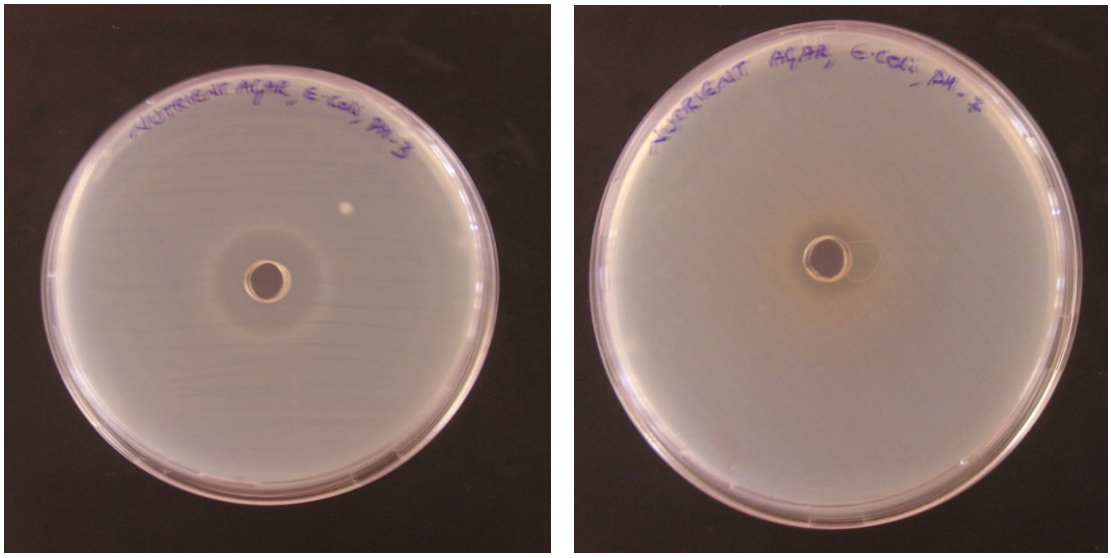


Plate 3. Maximum and minimum Zone of inhibition by *E. coli*

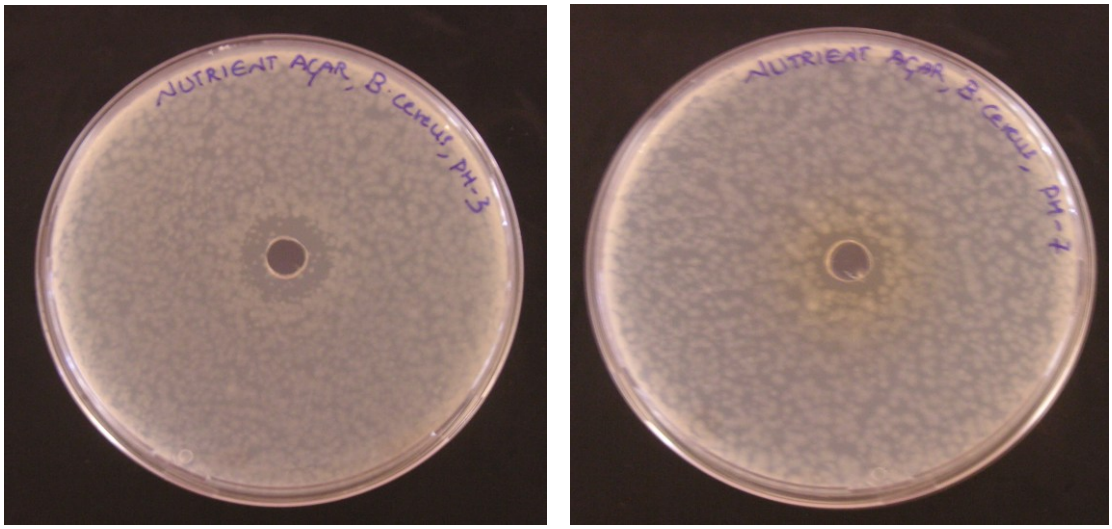


Plate 4. Maximum and minimum Zone of inhibition by *Bacillus cereus*

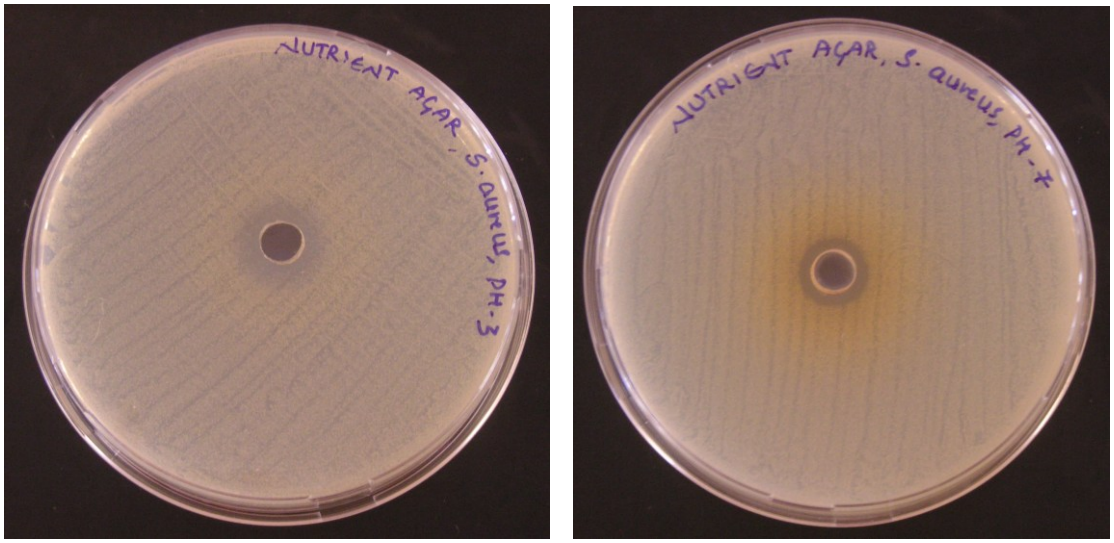


Plate 5. Maximum and minimum Zone of inhibition by *Staphylococcus aureus*

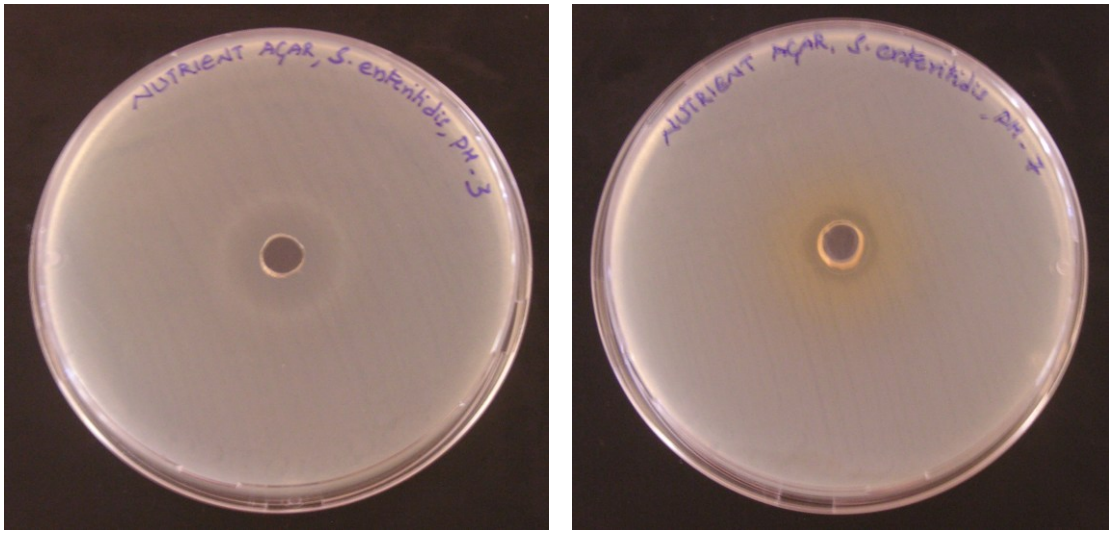


Plate 6. Maximum and minimum Zone of inhibition by *Salmonella enteritidis*

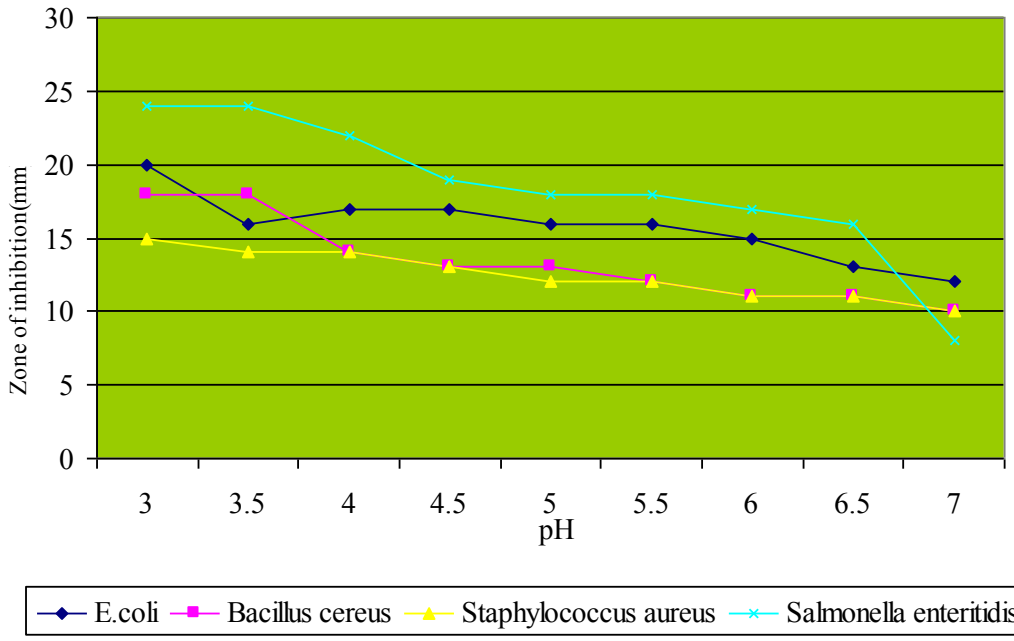


Fig. 3. Antagonistic activity of *L. acidophilus* MTCC 447 at varying pH

Table 6. Mean Score for the organoleptic qualities of the food mixtures

Treatment	Appearance	Colour	Flavour	Texture	Taste
T_1					
1	4.0	4.1	4.0	4.0	4.0
2	4.1	4.2	4.1	4.0	3.8
3	4.1	4.2	4.1	4.0	4.2
4	4.1	4.2	4.2	4.0	4.1
T_2					
1	4.0	4.3	3.9	4.1	4.1
2	4.0	4.0	3.9	3.8	4.1
3	4.2	4.2	4.0	4.3	4.0
4	4.0	4.0	3.9	3.9	4.0
T_3					
1	4.1	4.0	4.1	4.0	4.0
2	4.3	4.1	4.0	4.1	4.2
3	4.1	4.1	4.2	4.1	4.3
4	4.3	4.1	4.2	4.0	4.2
T_4					
1	4.1	4.1	3.9	3.8	4.0
2	4.0	4.0	4.1	4.0	4.0
3	4.1	4.1	4.1	4.0	4.0
4	4.1	4.0	4.1	4.0	4.0
T_5					
1	4.2	4.1	4.2	4.1	4.1
2	3.9	4.3	4.0	3.9	3.7
3	4.2	4.2	4.0	4.1	4.0
4	4.1	4.0	4.0	4.2	4.2
T_6					
1	4.0	4.0	4.2	4.2	4.2
2	3.9	4.2	4.1	4.2	3.9
3	4.1	4.1	4.1	4.3	4.2
4	4.1	4.1	4.3	4.2	4.0
T_7					
1	4.2	4.2	4.3	4.2	4.3
2	4.3	4.3	4.4	4.3	4.5
3	4.4	4.4	4.4	4.2	4.4
4	4.3	4.5	4.3	4.3	4.4
T_8					
1	4.2	4.1	4.2	3.8	4.0
2	4.2	4.2	4.0	4.0	4.1
3	4.3	4.2	4.1	4.0	4.0
4	4.1	4.2	4.1	4.0	4.0

Table 6 continued

Treatment	Appearance	Colour	Flavour	Texture	Taste
T_9					
1	4.2	4.3	4.2	4.0	4.2
2	4.1	4.3	4.0	4.2	4.2
3	4.2	4.3	4.0	4.2	4.2
4	4.5	4.3	4.2	4.2	4.1
T_{10}					
1	4.0	4.1	4.0	3.9	4.2
2	3.9	4.1	3.9	3.9	4.1
3	4.0	4.3	4.3	4.1	4.3
4	4.1	4.3	4.3	3.9	4.2
T_{11}					
1	4.0	4.0	4.0	4.0	3.9
2	4.2	4.2	4.2	4.0	3.9
3	4.0	4.1	4.2	4.1	4.2
4	4.2	4.2	4.2	4.1	4.2
T_{12}					
1	4.0	4.1	3.6	4.2	3.5
2	4.0	4.2	4.2	4.1	3.9
3	4.1	4.2	3.7	4.2	4.1
4	4.1	4.2	3.7	4.1	4.0
T_{13}					
1	4.3	4.2	4.0	4.2	3.7
2	4.3	4.2	4.1	4.1	3.7
3	4.3	4.1	4.1	4.2	3.9
4	4.5	4.0	4.3	4.2	4.2
T_{14}					
1	4.0	4.1	3.5	3.9	3.7
2	4.0	4.2	3.9	3.9	3.4
3	4.0	4.1	4.0	3.9	4.1
4	4.0	4.2	4.2	3.9	4.1

As revealed in the table 6, in treatment T_1, variation 1 had the least mean score for appearance (4.0), colour (4.1) and flavour (4.0) when compared to the other variations. There was no difference in the texture of the variations but mean score for taste was least in variation 2 (3.8). Maximum mean score for taste was for variation 3 but for flavour it was in variation 4 (4.2).

In T_2, variation 3 obtained the maximum score for appearance (4.2), flavour (4.0) and texture (4.3), but for colour, variation1 recorded the highest (4.3).There was no difference in the taste of variation 1 and 2 (4.1) but variations 3 and 4 showed less scores for taste (4.0)

In treatment T_3, appearance and colour of variation 2 and 4 scored the highest but, for flavour , texture and taste , highest score was for variation 3.

In treatment T_4, variation 2 had the least score for appearance (4.0) and colour (4.0) but the least score for flavour and texture was for variation 1.There was no difference in the mean score for taste among the variations in T4.

In T_5, quality aspects varied with the variations. Variation 2 showed the least score for appearance (3.9), but showed the maximum score for colour (4.3). Mean score for flavour was maximum for variation 1 (4.2), and variation 4 obtained the maximum for texture (4.2) and taste (4.2)

In T_6, variation 4 gained maximum score for appearance (4.1) and flavour (4.3), whereas variation 3 obtained maximum score for texture (4.3) and taste (4.2).

In T_7, variation 3 obtained the highest score for appearance (4.4) and flavour (4.4).Variation 4 had the highest score for colour (4.5), but variation 2 got the highest score for flavour (4.4), texture (4.3) and taste (4.5)

Among the variations in T_8, variation 3 showed the maximum score for appearance (4.3), colour (4.2) and texture (4.0). Flavour score was maximum for variation 1 (4.2), but the score for texture was the least (3.8). With regard to taste, variation 2 scored the highest (4.1).

In T_9, variation 4 scored highest for qualities like appearance (4.5), colour (4.3), flavour (4.3) and texture (4.2) but the least score for taste (4.1).

In T_{10}, variation 3 obtained maximum score for colour (4.3), flavour (4.3), texture (4.1) and taste (4.3).

In T_{11}, variation 4 showed the highest score for appearance (4.2), colour (4.2), flavour (4.2), texture (4.1) and taste (4.2).

In T_{12}, variation 3 scored highest for appearance (4.1), colour (4.2), texture (4.2) and taste (4.1) but flavour was highest in variation 2 (4.2).

Among the variations in T_{13}, variation 4 obtained maximum socre for appearance (4.5), flavour (4.3), texture (4.2) and taste (4.2), but for colour, it was in variation 1 and 2 (4.2).

In T_{14}, there was no difference in the mean score for appearance (4.0) and texture (3.9) among variations, but for variation 4 other qualities like colour (4.2), flavour (4.2) and taste (4.1) recorded the highest score.

The mean score obtained for the organoleptic qualities of each variation of the 14 treatments were statistically analysed using Kendall's coefficient of concordance and the mean ranks were worked out.

An index was worked out for each variation using mean ranks obtained through Kendall's test for all the five parameters [Appearance (X_1), colour(X_2), flavour(X_3), texture(X_4), and taste(X_5),) as $W_1X_1 + W_2X_2 + W_3X_3 + W_4X_4$ and W_5X_5 where W_1, W_2, W_3, W_4 and W_5 were weights assigned to the different ranks under taste, texture, flavour, colour and appearance as 5, 4, 3, 1.5 and 1.5 respectively. The weights were assigned logically. The attributes of weight assigned will in no way alter the sequential ordering of the combinations. The results are presented in Table 7.

Table 7. Mean score and index for the organoleptic qualities of the food mixtures

Treatment	**Appearance** (1.5)	**Colour** (1.5)	**Flavour** (3)	**Texture** (4)	**Taste** (5)	**Total Index**
T_1						
1	37.58 (4.0)	39.83 (4.1)	79.20 (4.0)	107.40 (4.0)	133.25 (4.0)	397.26
2	41.10 (4.1)	43.73 (4.2)	87.15 (4.1)	107.40 (4.0)	123.25 (3.8)	402.63
3	41.75 (4.1)	43.95 (4.2)	87.15 (4.1)	107.40 (4.0)	157.75 (4.2)	438.00
4	41.10 (4.1)	43.73 (4.2)	93.75 (4.2)	107.40 (4.0)	157.75 (4.1)	**443.73**
T_2						
1	37.58 (4.0)	47.93 (4.3)	72.00 (3.9)	117.80 (4.1)	145.75 (4.1)	421.06
2	38.33 (4.0)	36.30 (4.0)	72.15 (3.9)	91.00 (3.8)	146.75 (4.1)	384.53
3	46.95 (4.2)	43.95 (4.2)	80.25 (4.0)	137.40 (4.3)	135.75 (4.0)	**444.30**
4	38.63 (4.0)	36.75 (4.0)	73.50 (3.9)	102.00 (3.9)	140.50 (4.0)	391.38
T_3						
1	41.63 (4.1)	36.75 (4.0)	88.80 (4.1)	108.80 (4.0)	132.50 (4.0)	408.48
2	50.10 (4.3)	40.88 (4.1)	72.15 (4.0)	117.00 (4.1)	157.75 (4.)	437.88
3	41.63 (4.1)	40.28 (4.1)	95.40 (4.2)	117.20 (4.1)	171.25 (4.3)	**465.76**
4	50.10 (4.3)	40.88 (4.1)	94.50 (4.2)	109.60 (4.0)	158.25 (4.2)	453.33
T_4						
1	41.63 (4.1)	39.98 (4.1)	72.30 (3.9)	88.40 (3.8)	133.25 (4.0)	375.56
2	37.58 (4.0)	35.85 (4.0)	87.45 (4.1)	107.60 (4.0)	132.25 (4.0)	400.73
3	41.63 (4.1)	39.98 (4.1)	87.45 (4.1)	107.40 (4.0)	133.25 (4.0)	409.71
4	41.78 (4.1)	36.45 (4.0)	87.45 (4.1)	108.40 (4.0)	149.00 (4.0)	**423.08**
T_5						
1	45.38 (4.2)	39.83 (4.1)	86.25 (4.1)	116.00 (4.2)	145.50 (4.1)	432.96
2	34.13 (3.9)	47.93 (4.3)	79.35 (4.1)	99.20 (4.0)	98.50 (3.9)	359.11
3	45.53 (4.2)	43.95 (4.2)	78.30 (4.1)	117.20 (4.0)	136.75 (4.1)	421.73
4	41.48 (4.1)	37.35 (4.0)	79.50 (4.1)	127.60 (4.0)	159.00 (4.2)	**444.93**

Table 7 continued

Treatment	Appearance (1.5)	Colour (1.5)	Flavour (3)	Texture (4)	Taste (5)	Total Index
T_6						
1	38.33 (4.0)	37.65 (4.0)	94.05 (4.2)	128.00 (4.2)	156.75 (4.2)	454.78
2	34.13 (3.9)	43.95 (4.2)	86.85 (4.1)	128.00 (4.2)	122.00 (3.9)	414.93
3	41.25 (4.1)	39.60 (4.1)	87.45 (4.1)	133.60 (4.3)	156.75 (4.2)	**458.65**
4	41.55 (4.1)	39.98 (4.1)	87.15 (4.3)	126.24 (4.2)	132.75 (4.0)	427.67
T_7						
1	45.68 (4.2)	44.10 (4.2)	103.50 (4.3)	129.00 (4.2)	173.25 (4.3)	495.53
2	48.83 (4.3)	47.76 (4.3)	108.60 (4.4)	136.00 (4.3)	183.50 (4.5)	524.69
3	53.48 (4.4)	52.28 (4.4)	111.45 (4.4)	129.00 (4.2)	192.00 (4.4)	**538.21**
4	49.58 (4.3)	56.10 (4.5)	101.85 (4.3)	135.40 (4.3)	181.75 (4.4)	524.68
T_8						
1	46.20 (4.2)	39.83 (4.1)	95.70 (4.2)	89.20 (3.8)	133.75 (4.0)	404.68
2	45.60 (4.2)	43.95 (4.2)	79.20 (4.0)	108.60 (4.0)	146.50 (4.1)	423.85
3	49.73 (4.3)	45.00 (4.2)	87.45 (4.1)	110.40 (4.0)	134.75 (4.0)	**427.33**
4	42.90 (4.1)	43.95 (4.2)	87.15 (4.1)	108.20 (4.0)	133.25 (4.0)	415.45
T_9						
1	32.05 (4.2)	49.58 (4.3)	95.55 (4.2)	110.00 (4.0)	160.00 (4.2)	447.18
2	42.00 (4.1)	48.08 (4.3)	79.50 (4.0)	129.00 (4.2)	156.00 (4.2)	454.58
3	45.30 (4.2)	48.23 (4.3)	79.50 (4.0)	130.60 (4.2)	158.50 (4.2)	462.13
4	57.60 (4.5)	56.55 (4.3)	95.55 (4.2)	128.60 (4.2)	150.00 (4.1)	**488.3**
T_{10}						
1	38.18 (4.0)	41.10 (4.1)	78.90 (4.0)	98.60 (3.9)	156.75 (4.2)	413.53
2	30.90 (3.9)	39.83 (4.1)	73.05 (3.9)	98.60 (3.9)	143.75 (4.1)	386.13
3	37.58 (4.0)	47.93 (4.3)	102.60 (4.3)	117.00 (4.1)	170.00 (4.3)	**475.11**
4	41.48 (4.1)	47.93 (4.3)	103.65 (4.3)	100.00 (3.9)	160.00 (4.2)	453.06

Table 7 continued

Treatment	Appearance (1.5)	Colour (1.5)	Flavour (3)	Texture (4)	Taste (5)	Total Index
T_{11}						
1	34.65 (4.0)	36.30 (4.0)	80.55 (4.0)	108.20 (4.0)	120.50 (3.9)	380.2
2	45.30 (4.2)	43.80 (4.2)	94.05 (4.2)	111.20 (4.0)	121.50 (3.9)	415.85
3	37.73 (4.0)	39.83 (4.1)	97.65 (4.2)	116.80 (4.1)	158.50 (4.2)	450.51
4	45.60 (4.2)	43.80 (4.2)	95.55 (4.2)	108.20 (4.1)	158.75 (4.2)	**451.90**
T_{12}						
1	38.63 (4.0)	39.98 (4.1)	48.90 (3.6)	124.60 (4.2)	72.25 (3.5)	324.36
2	39.08 (4.0)	45.75 (4.2)	57.15 (4.2)	103.00 (4.1)	125.75 (3.9)	370.73
3	41.48 (4.1)	43.95 (4.2)	57.00 (3.7)	127.40 (4.2)	149.00 (4.1)	**418.83**
4	42.08 (4.1)	43.95 (4.2)	57.15 (3.7)	114.80 (4.1)	137.25 (4.0)	395.23
T_{13}						
1	34.35 (4.3)	43.95 (4.2)	79.20 (4.0)	127.60 (4.2)	101.00 (3.7)	386.1
2	49.13 (4.3)	43.73 (4.2)	85.80 (4.1)	117.40 (4.1)	97.00 (3.7)	393.06
3	49.28 (4.3)	39.98 (4.1)	87.30 (4.1)	127.60 (4.2)	122.50 (3.9)	426.66
4	57.38 (4.5)	35.85 (4.0)	102.75 (4.3)	127.00 (4.2)	160.00 (4.2)	**482.98**
T_{14}						
1	37.58 (4.0)	39.83 (4.1)	65.55 (3.5)	98.60 (3.9)	114.75 (3.7)	356.31
2	37.95 (4.0)	43.95 (4.2)	72.60 (3.9)	98.40 (3.9)	75.00 (3.4)	327.9
3	38.93 (4.0)	39.98 (4.1)	79.20 (4.0)	98.40 (3.9)	159.25 (4.1)	415.76
4	38.93 (4.0)	39.83 (4.2)	95.70 (4.2)	98.60 (3.9)	160.00 (4.1)	**433.06**

(Figures in parenthesis indicate mean score)

The index worked out helped to identify the best variation in each treatment by considering all the organoleptic qualities. The best variation with maximum index and the combination of food ingredients in the selected variation in each treatment is presented in Table.8

Table 8. Selected food combinations in each treatment

Food mixtures (Treatments)	Combinations (percent)	Variation No:
T_1	B-70, DS-20, M-10	4
T_2	B-60, DS-20, P-20	3
T_3	B-60, DS-20, T-20	3
T_4	B-70, GG-20, M-10	4
T_5	B-70, GG-20, P-10	4
T_6	B-60, GG-20, T-20	3
T_7	B-60, DS-20, M-10, P-10	3
T_8	B-60, DS-20, M-10, T-10	3
T_9	B-70, DS-,20, P-5, T-5	4
T_{10}	B-60, GG-20, M-10, P-10	3
T_{11}	B-70, GG-20, M-5, T-5	4
T_{12}	B-60, GG-20, P-10, T-10	3
T_{13}	B-70, DS-20, M-3.34, P-3.34, T-3.34	4
T_{14}	B-70, GG-20, M-3.34, P-3.34, T-3.34	4

B- Banana, DS- Defatted soya flour, GG- Green gram flour, M- Mango, T-Tomato, P-Papaya

Among the 14 fourteen food mixtures, variation 4 was the best in T_1, T_4, T_5, T_9, T_{11}, T_{13} and T_{14} whereas variation 3 was found to be the best in T_2, T_3, T_6, T_7, T_8, T_{10} and T_{12} which obtained the maximum total index.

Among the selected variations, variation 4 contained 70 percent banana flour, whereas variation 3, contained 60 per cent banana flour. Twenty percent defatted soya flour was found acceptable in T_1, T_2, T_3, T_7, T_8, T_9 and T_{13} whereas 20 percent green gram flour was found acceptable in T_4, T_5, T_6, T_{10}, T_{11}, T_{12} and T_{14}. Fruit pulps never exceeded 20 percent in any of the acceptable combinations. A combination of mango, papaya and tomato in equal proportions constituting a total of about 10 percent was the acceptable variation in T_{13} and T_{14} but the only difference between these two treatments was 20 percent green gram flour in T_{14} and 20 percent defatted soya flour in T_{13}. A combination of 10 percent papaya pulp and 10 percent mango pulp was found to be acceptable in T_{10} and T_7 whereas in T_8 this was 10 percent mango and 10 percent tomato. Mango, tomato combination was acceptable in T_{11} but only at 5 percent level

each. A combination of papaya and tomato at 10 percent level each, was found to be acceptable in T_{12} whereas in T_9, acceptability was for 5 percent papaya and 5 percent tomato pulp.

In T_1 and T_4, mango pulp at 10 percent level was found to be acceptable. In T_2 and T_5, papaya pulp at 20 percent and 10 percent level respectively were acceptable. In T_3 and T_6, 20 percent tomato pulp was found to be acceptable.

4.4. Optimisation of variables for fermentation of food mixtures

Optimisation process was carried out using *L. acidophilus* for fermentation, and total viable count in the product was maximised while variables like substrate concentration, quantity of the inoculum, time of incubation, pH and temperature were kept at acceptable levels and the results are given below.

4.4.1Optimisation of substrate concentration

Three different quantities of each food mixtures (25, 50 and 75g) were taken for optimising the substrate concentration. After fermentation, the freeze dried samples were enumerated for viable counts of *L. acidophilus*. The results are presented in Table 9.

As revealed in Table 9, 10^7dilutions of all the treatments were compared, each with three substrate concentration. In all the treatments, substrate concentration of 25g showed maximum viable count of *L. acidophilus* ranging from 39 to 76 x 10^7cfu/g (Fig 4). When expressed in log cfu/g, the viable count of treatments with a substrate concentration of 25g ranged between 8.59 to 8.88 log cfu/g.

Table 9. Viable count (x10^7cfu/g) of *L. acidophilus* in food mixtures with different substrate concentrations

Quantity of substrate (g) / Treatments (Food mixtures)	25	50	75
	Viable count		
T_1	53	30	TFTC
T_2	39	TFTC	0
T_3	71	32	TFTC
T_4	67	33	TFTC
T_5	43	28	0
T_6	76	38	TFTC
T_7	48	TFTC	TFTC
T_8	65	58	TFTC
T_9	47	27	TFTC
T_{10}	40	TFTC	TFTC
T_{11}	70	35	TFTC
T_{12}	61	31	TFTC
T_{13}	53	31	TFTC
T_{14}	48	30	TFTC

All values are mean of 3 independent enumerations
TFTC- Too few to count

4.4.2. Optimisation of pH

Twenty five gram of each food mixture was taken for further standardisation procedures. pH of the substrate was adjusted to 3.5, 4.5, 5.5 and 6.5 using citric acid (20 percent). The substrates with pH 3.5 got hydrolysed to a thin watery consistency after fermentation so it was not possible for freeze drying. Hence substrate at pH 3.5 was eliminated. Other samples were freeze dried and viable counts were enumerated by pour plate method and the results are presented in Table 10.

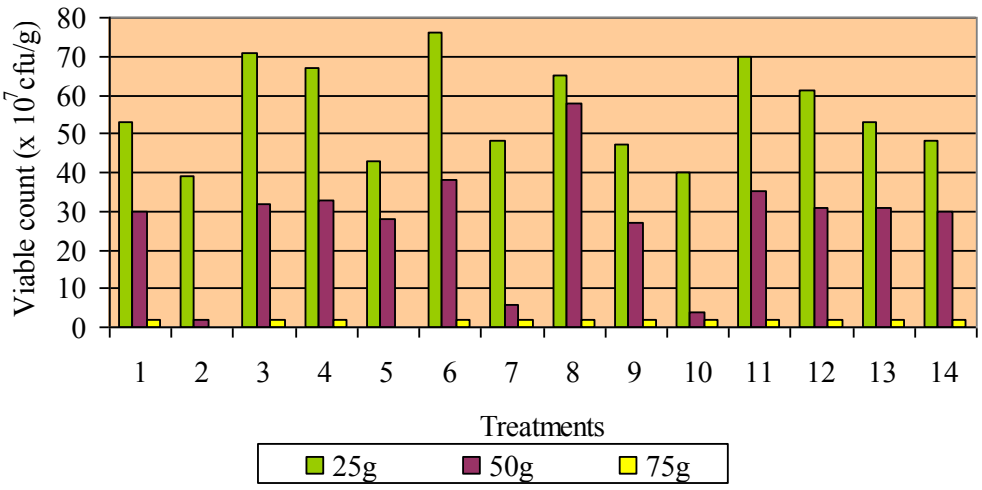


Fig. 4. Viable count of *L. acidophilus* in food mixtures with different substrate concentration

Table 10. Viable count of *L. acidophilus* (x 10^7cfu/g) in food mixtures with different pH levels

pH / Treatments	4.5	5.5	6.5
T_1	63	37	TFTC
T_2	40	TFTC	0
T_3	99	43	TFTC
T_4	71	43	TFTC
T_5	49	TFTC	0
T_6	103	47	TFTC
T_7	51	TFTC	0
T_8	65	61	TFTC
T_9	53	27	TFTC
T_{10}	51	30	TFTC
T_{11}	93	46	TFTC
T_{12}	84	35	TFTC
T_{13}	67	31	TFTC
T_{14}	56	30	TFTC

All values are mean of 3 independent enumerations.
TFTC- Too few to count

10^7dilutions of all the treatments were compared, each with different pH levels. In all the treatments, a pH of 4.5 showed maximum viable count of *L. acidophilus* ranging from 40 to 103 x 10^7 cfu/g (Fig 5). When expressed in log cfu/g, the viable count of the treatments with a substrate concentration of 25g at pH 4.5 ranged between 8.60 to 9.01 log cfu/g.

4.4.3. Optimisation of temperature for fermentation

Twenty five gram of each food mixtures was taken and pH was adjusted to 4.5. After fermentation, incubation was done in 37ºC, 41ºC and 45ºC. The freeze dried samples were enumerated for total viable count of *L. acidophilus* and the results are presented in the Table 11.

Table 11. Viable count of *L. acidophilus* (x10^7cfu/g) in food mixtures with different temperatures for incubation

Temperature (°C) / Treatments	37	41	45
	Viable count		
T_1	65	32	0
T_2	41	35	0
T_3	97	32	0
T_4	71	38	TFTC
T_5	49	41	0
T_6	99	36	0
T_7	37	26	0
T_8	67	37	TFTC
T_9	57	31	0
T_{10}	50	33	0
T_{11}	80	38	TFTC
T_{12}	63	41	0
T_{13}	65	31	0
T_{14}	51	30	0

All values are mean of 3 independent enumerations.
TFTC- Too few to count

10^7dilutions of all the treatments were compared, each incubated in different temperatures. In all the treatments, a temperature of 37 °C showed maximum viable count of *L.* acidophilus ranging from 37 to 99 x10^7cfu/g (Fig 6). When expressed in log cfu/g, the viable count of treatments with a substrate concentration of 25g with pH 4.5 incubated at temperature 37 ° C ranged between 8.54 to 8.99 log cfu/g.

4.4.4. Optimisation of time of incubation

Twenty five gram of each food mixture after adjusting the pH at 4.5, was fermented at 37° C for three different periods, such as 18 hours, 24hours and 30 hours. The freeze dried samples were enumerated for total viable count and the results are presented in the table 12.

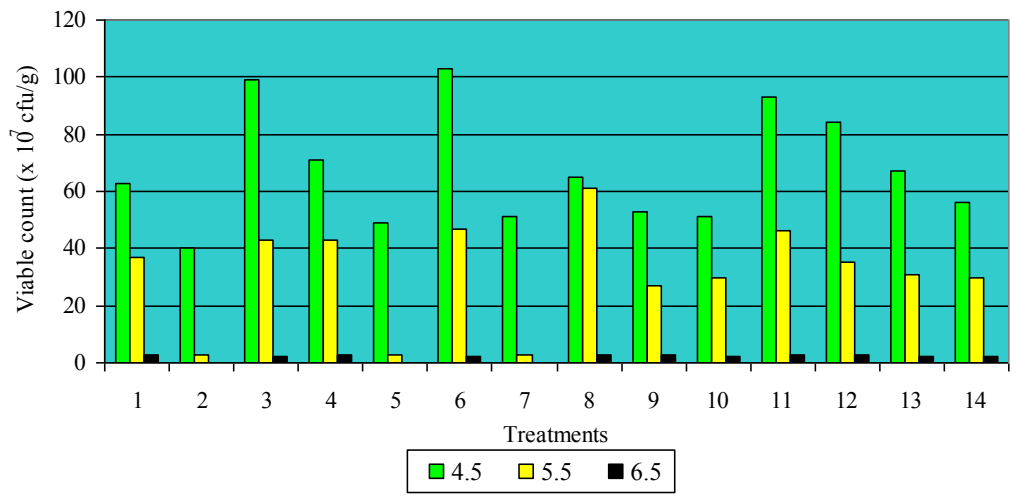


Fig.5.Viable count of *L.acidophilus* in food mixtures with different pH levels

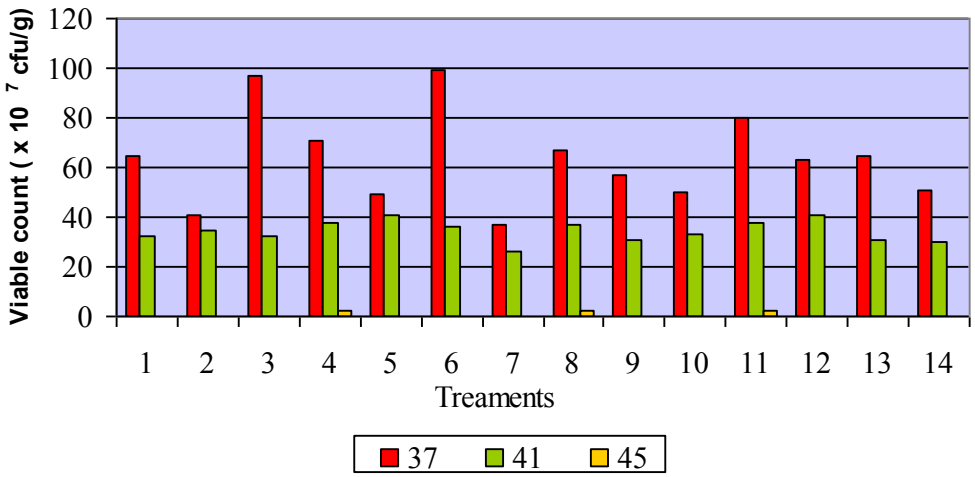


Fig.6.Viable count of *L.acidophilus* in food mixtures with different temperatures(^{o} C) for incubation

Table 12. Viable count of *L. acidophilus* (x10^7cfu/g) in food mixtures with different time of incubation.

Time (h) / Treatments	18	24	30
	Viable count		
T_1	32	63	27
T_2	30	40	TFTC
T_3	34	99	21
T_4	39	72	42
T_5	41	49	0
T_6	40	100	30
T_7	26	40	TFTC
T_8	30	66	TFTC
T_9	31	54	TFTC
T_{10}	33	45	TFTC
T_{11}	40	89	30
T_{12}	45	88	TFTC
T_{13}	21	56	TFTC
T_{14}	33	67	TFTC

All values are mean of 3 independent enumerations
TFTC- Too few to count

10^7dilutions of all the treatments were compared, each incubated for different intervals of time. In all the treatments, 24 hour of incubation showed maximum viable count of *L. acidophilus* ranging from 40 to 100 x10^7 cfu/g (Fig 7). When expressed in log cfu/g, the viable count of treatments with a substrate concentration of 25g at pH 4.5 incubated at 37 ^{0} C for 24 hours ranged between 8.60 to 9.00 log cfu/g.

4.4.5. Optimisation of inoculum concentration for fermentation

Twenty five gram of each food mixture after adjusting the pH at 4.5 was inoculated with three different concentration of inoculum (*L. acidophilus*) i.e. 100µl (107x10^6 cfu/ml), 200µl (116x10^6 cfu/ml) and 300µl (119 x10^6 cfu/ml) and was kept for incubation at 37ºC for 24 hours. The freeze dried samples were enumerated for the total viable count and the results are presented in the table 13. (Fig 8).

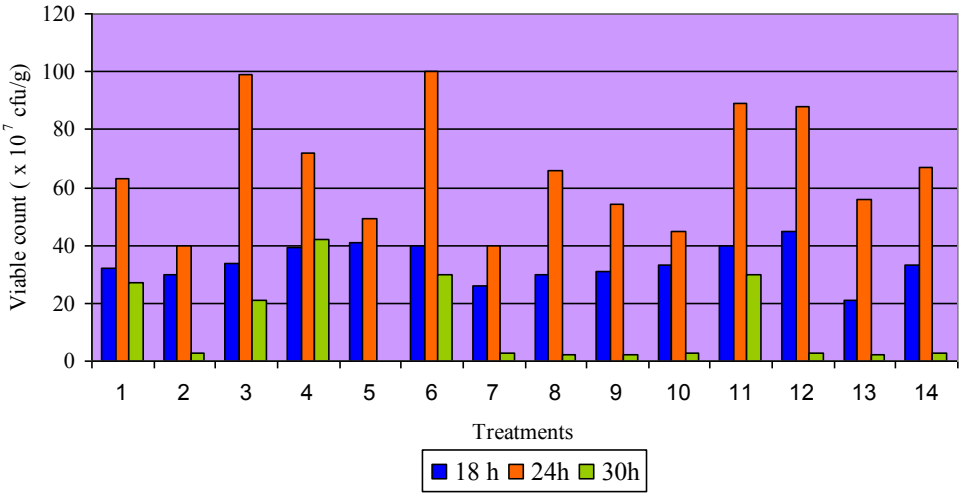


Fig.7. Viable count of *L.acidophillus* in food mixtures with different time of incubation

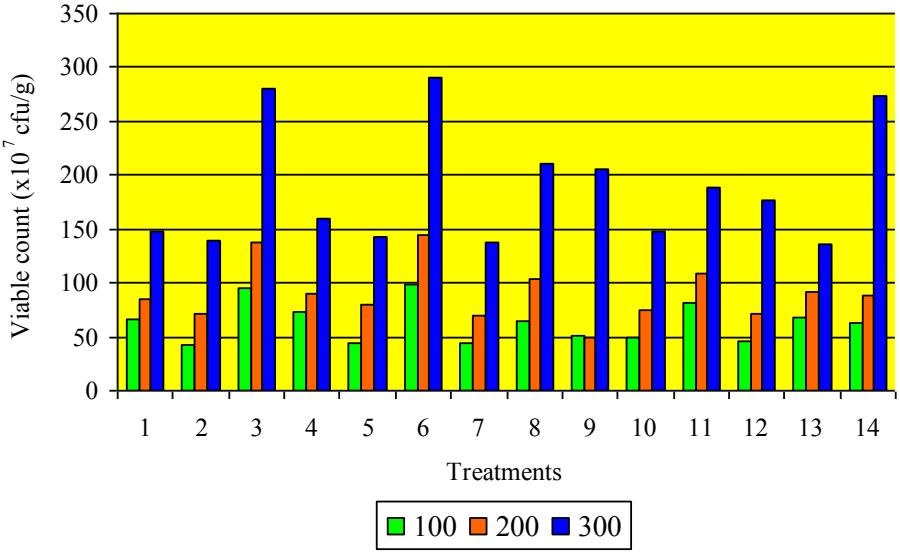


Fig. 8. Viable count of *L.acidophillus* in food mixtures with different concentration of inoculum(ul)

Table 13. Viable count of *l. acidophilus* (x10^7cfu/g) in food mixtures with different concentrations of inoculum.

Inoculum(µl) / Treatments	100	200	300
	Viable count		
T_1	67	85	148
T_2	42	72	139
T_3	95	137	281
T_4	73	90	159
T_5	45	80	143
T_6	99	144	291
T_7	45	69	137
T_8	65	104	210
T_9	51	50	205
T_{10}	49	75	147
T_{11}	81	108	189
T_{12}	46	71	176
T_{13}	68	91	136
T_{14}	63	88	273

All values are mean of 3 independent enumerations
TFTC- Too few to count

Total viable count was maximum with 300µl of the inoculum in all the treatments which ranged from 136 to 291 x 10^7 cfu/g. (Fig 8)

Thus for all the treatments, fermentation with 25g of the substrate at pH 4.5, inoculated with 300µl (119 x10^6 cfu/ml) and incubated at 37ºC for 24 hours gave the maximum total viable count of *L. acidophilus* ranging from 9.13 to 9.46 log cfu/g and this total viable count is above the desired level of probiotic organism as recommended by Shah (1995).

4.5. Development of fermented food mixtures (FFM).

Fermented food mixtures (FFM) were prepared with the optimum variables. Two control samples viz; fermented food mixtures without autoclaving and

autoclaved and unfermented food mixtures (UFFM) were also developed for each treatment.

After 24hours of incubation, all the fermented samples without autoclaving were heavily contaminated and hence these samples were discarded (Plate 7). Rest of the fermented as well as the unfermented samples (controls) were freeze dried and were used for further studies (Plate 8).

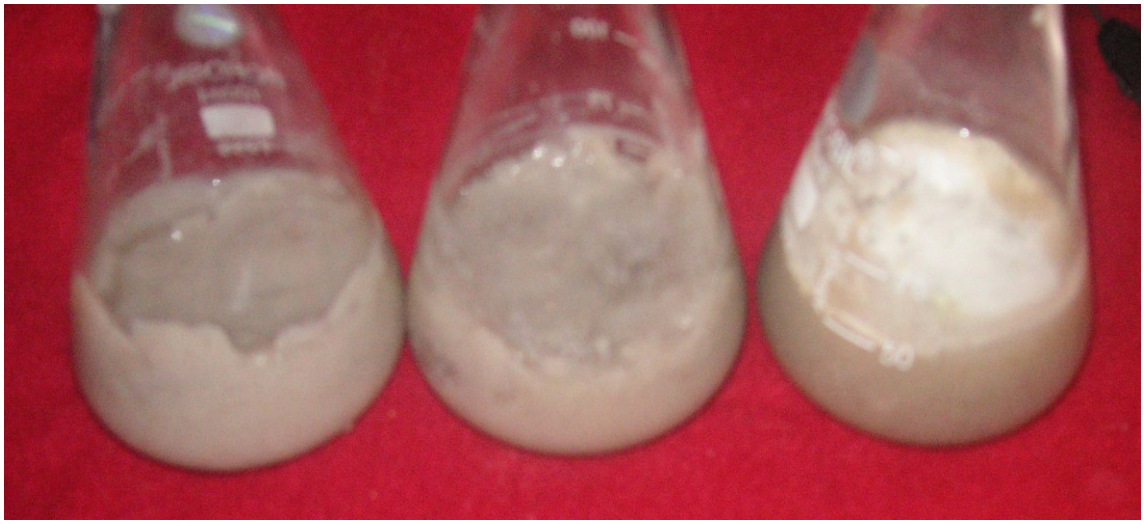
4.5.1. Chemical analysis of the fermented and unfermented food mixtures.

Table.14. Moisture, titrable acidity, protein, β carotene and crude fiber in fermented food mixtures

Treatments	Moisture (%)	Titrable Acidity (g lactic acid /100 g)	Protein (g / 100g)	β carotene (µg/ 100g)	Fiber (g/100g)
T_1	1.80 ^{a}	2.50 ^{a}	9.43 ^{b}	563.52 ^{a}	Trace
T_2	1.85 ^{bc}	2.50 ^{a}	8.80 ^{d}	509.06 ^{b}	Trace
T_3	1.84 ^{bc}	2.54 ^{a}	9.81 ^{a}	470.90 ^{d}	Trace
T_4	1.85 ^{bc}	2.56 ^{a}	6.66 ^{e}	395.61 ^{c}	Trace
T_5	1.81 ^{a}	2.66 ^{a}	6.65 ^{e}	324.91 ^{e}	Trace
T_6	1.86 ^{bc}	2.68 ^{a}	6.39 ^{f}	327.17 ^{b}	Trace
T_7	1.85 ^{bc}	2.54 ^{a}	9.13 ^{c}	543.66 ^{c}	Trace
T_8	1.85 ^{bc}	2.54 ^{a}	9.45 ^{b}	515.08 ^{b}	Trace
T_9	1.84 ^{bc}	2.53 ^{a}	9.09 ^{c}	483.36 ^{d}	Trace
T_{10}	1.84 ^{bc}	2.63 ^{a}	6.32 ^{f}	377.13 ^{e}	Trace
T_{11}	1.85 ^{bc}	2.67 ^{a}	5.98 ^{g}	283.60 ^{e}	Trace
T_{12}	1.82 ^{ab}	2.68 ^{a}	6.69 ^{e}	291.34 ^{e}	Trace
T_{13}	1.83 ^{ab}	2.55 ^{a}	8.79 ^{d}	508.81 ^{b}	Trace
T_{14}	1.84 ^{bc}	2.67 ^{a}	6.32 ^{f}	301.73 ^{f}	Trace

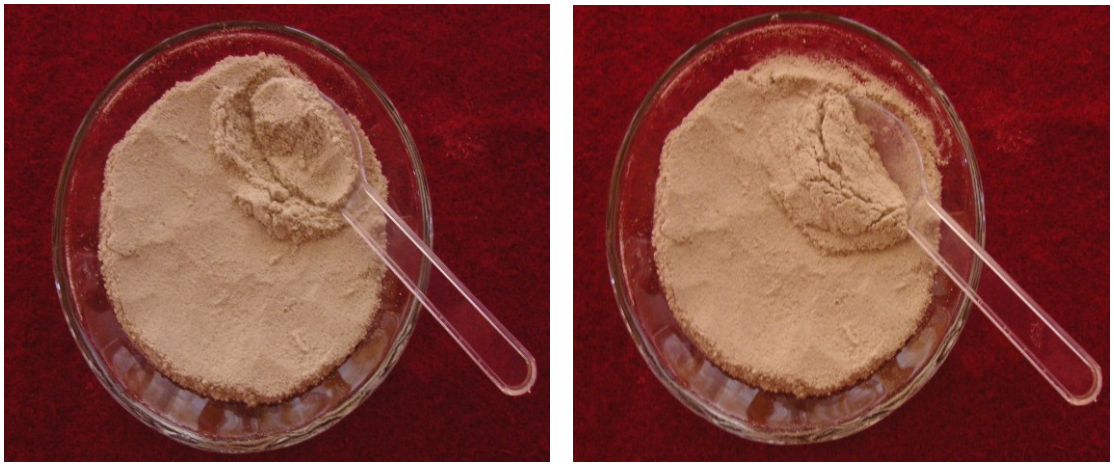
Values are mean of three independent determinations
Values with same superscripts do not have significant difference
DMRT column wise comparison

As revealed in table 14, between the fermented samples moisture content varied between 1.80 and 1.86 percent. There was no significant variation in the moisture content of treatments T_1, T_5, T_{12} and T_{13}. T_1 showed the least moisture content among



Unfermented food mixture | Fermented food mixture | Fermented food mixture without autoclaving

Plate 7. Contamination in fermented samples without autoclaving



Fermented food mixture | Unfermented food mixture

Plate 8. Freeze dried unfermented and fermented food mixtures

treatments. Titrable acidity was between 2.50 to 2.68 (g lactic acid/100g) the maximum being in T_6 and T_{12}, but no significant variation in acidity between treatments. Maximum protein content was observed in T_3 (9.81g/100g), whereas T_{10} and T_{14} has the least protein (6.32g/100g). There was a significant variation in the protein content of different treatments. β carotene content ranged between 301.73 to 563.52 µg/100g which showed a significant variation between treatments. None of the fermented samples contained detectable amounts of crude fiber. Thus there observed a significant variation in moisture, titrable acidity, protein and β carotene content between the 14 fermented samples.

Table 15. Moisture, titrable acidity, crude protein, β carotene and crude fiber in unfermented food mixture

Treatments	Moisture (%)	Titrable Acidity (g lactic /100 g)	Protein (g /100g)	β carotene (µg/100g)	Fiber (g/100g)
T_1	1.80 ^{a}	1.26 ^{a}	7.37 ^{b}	567.49 ^{a}	0.83 ^{a}
T_2	1.84 ^{b}	1.27 ^{a}	6.83 ^{f}	511.35 ^{d}	0.81 ^{b}
T_3	1.84 ^{b}	1.25 ^{a}	7.65 ^{a}	470.62 ^{f}	0.82 ^{ab}
T_4	1.85 ^{b}	1.31 ^{a}	4.96 ^{h}	395.41 ^{g}	0.31 ^{d}
T_5	1.81 ^{ab}	1.31 ^{a}	5.13 ^{g}	325.94 ^{j}	0.33 ^{cd}
T_6	1.85 ^{b}	1.34 ^{a}	4.47 ^{l}	330.56 ^{i}	0.33 ^{c}
T_7	1.83 ^{ab}	1.28 ^{a}	7.04 ^{c}	545.07 ^{b}	0.81 ^{b}
T_8	1.83 ^{ab}	1.24 ^{a}	7.37 ^{b}	515.90 ^{c}	0.80 ^{b}
T_9	1.82 ^{ab}	1.23 ^{a}	7.01 ^{d}	484.40 ^{e}	0.81 ^{b}
T_{10}	1.83 ^{ab}	1.34 ^{a}	4.42 ^{i}	377.63 ^{h}	0.32 ^{cd}
T_{11}	1.83 ^{ab}	1.31 ^{a}	4.07 ^{l}	285.17 ^{m}	0.34 ^{c}
T_{12}	1.82 ^{ab}	1.31 ^{a}	4.75 ^{i}	295.18 ^{l}	0.33 ^{c}
T_{13}	1.85 ^{b}	1.23 ^{a}	6.92 ^{e}	513.11 ^{d}	0.83 ^{a}
T_{14}	1.84 ^{b}	1.33 ^{a}	4.13 ^{k}	302.57 ^{k}	0.34 ^{c}

Values are mean of three independent determinations
Values with same superscript do not have significant difference
DMRT column wise comparison

As shown in Table 15, in unfermented samples, moisture content varied between 1.81 to 1.85 percent and titrable acidity between 1.23 to 1.28 (g lactic acid /100 g). There was a wide variation in the protein content which varied from 4.07 to 7.65g/100g. T_3 contained the maximum protein and T_{11} the minimum. β carotene

content ranged between 285.17 to 567.49 µg/100g. Unfermented samples contained fiber which ranged from 0.31 to 0.83 g/100g. There was a significant variation in the moisture, acidity, protein, β carotene and crude fiber between treatments in the control samples also.

Fermented and unfermented treatments were statistically compared for their moisture, titrable acidity, protein and β carotene by applying independent sample't' test and is presented in table 16.

Table 16. Moisture, titrable acidity, crude protein, β carotene and crude fiber in FFM and UFFM

Methods	Moisture (%)	Titrable Acidity (g lactic acid /100 g)	Protein (g/100g)	β carotene (µg/100g)	Fiber (g/100g)
FFM	1.837	2.59	7.82	421.14	Trace
UFFM	1.831	1.27	5.87	422.75	0.57
Mean difference	0.0064	1.303	1.957	-1.612	-
t value	1.051	106.08	6.399	-0.74	-15.042
Significance	0.303	0.0001	0.0001	.941	-
	NS	S	S	NS	-

There was no significant difference in the moisture and β carotene of FFM and UFFM. The protein and titrable acidity in FFM were significantly high ($p< 0.05$). Crude fiber was significantly high in UFFM. The moisture, titrable acidity. protein and β carotene content of FFM and UFFM in depicted in Fig 9 to 12.

As shown in Table17, TSS ranged between 11.61 to 11.68 °Brix in FFM and the difference in TSS was not significant among the treatments. There was a significant variation in the starch, reducing sugar and total sugar in FFM. Starch content varied from 51.93g (T_{11}) to 55.06g (T_2). Reducing sugar varied from 2.11 to 3.07 g/100g, the maximum in T_1 which showed no significant variation with T_7. Total sugar was maximum in T_1 (5.64 g/100g) and minimum in T_{12} (4.76 g/100g). Thus there was a significant difference in starch, reducing sugar, and total sugars between the 14 FFM.

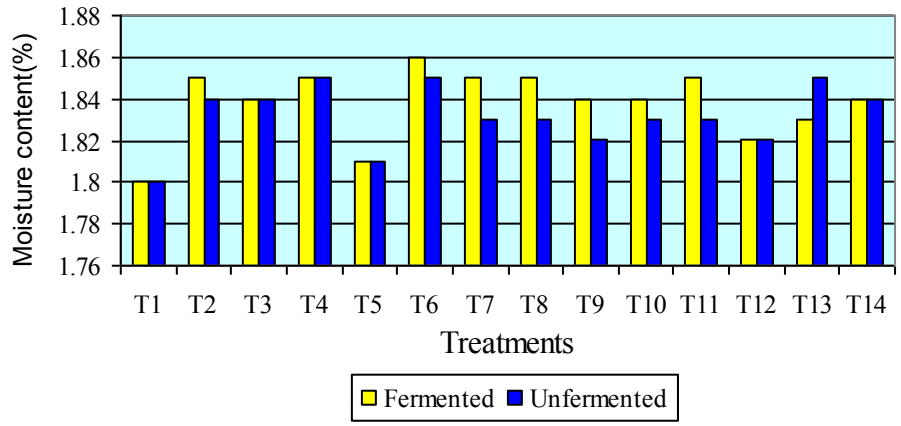


Fig. 9. Moisture content in fermented and unfermented food mixtures

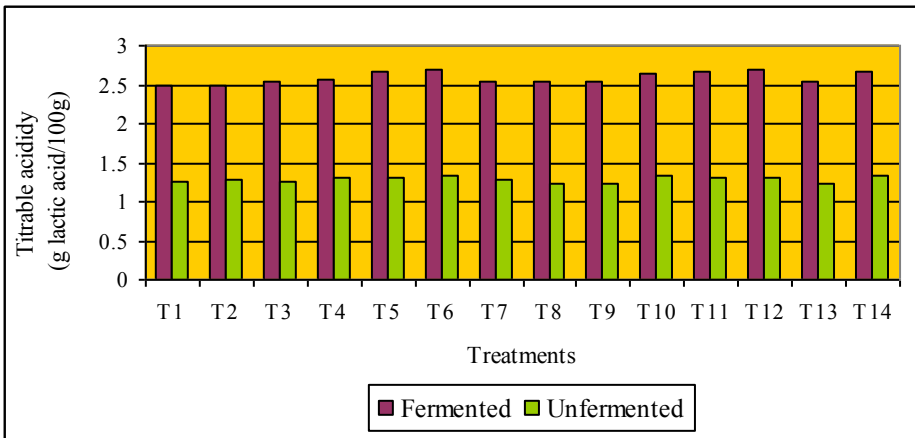


Fig. 10. Titrable acidity in fermented and unfermented food mixtures

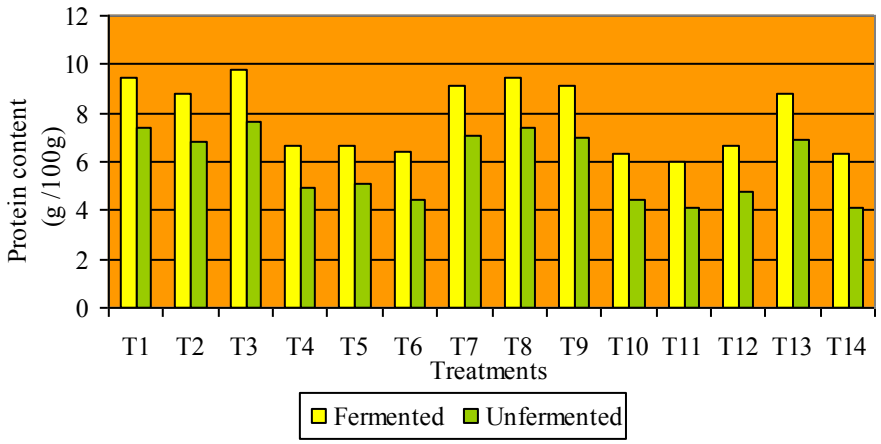


Fig. 11. Protein content in fermented and unfermented food mixtures

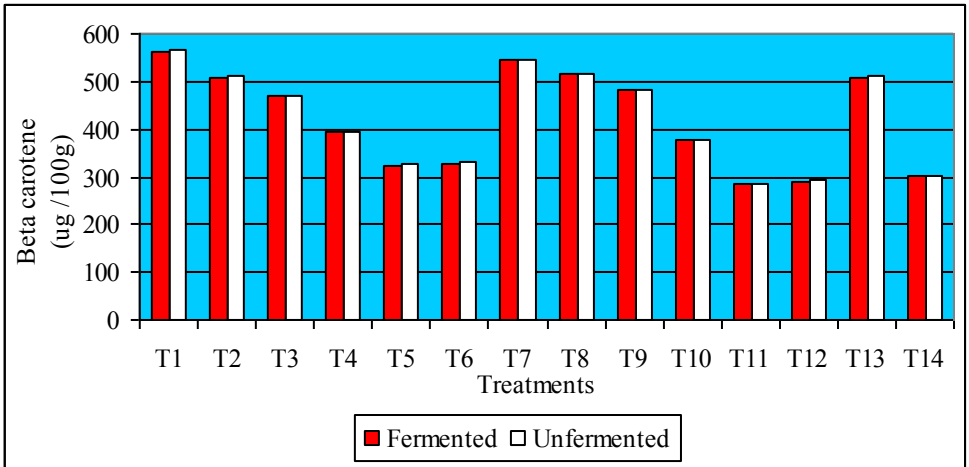


Fig. 12. Beta carotene in fermented and unfermented food mixtures

Table 17. TSS (°Brix), starch, reducing sugar and total sugars in fermented food mixtures (g/100g)

Treatments	TSS	Starch	Reducing Sugar	Total Sugars
T_1	11.63 ^{a}	54.77 ^{bc}	3.07 ^{a}	5.64 ^{a}
T_2	11.68 ^{a}	55.06 ^{a}	2.95 ^{c}	5.38 ^{c}
T_3	11.65 ^{a}	54.57 ^{d}	2.79 ^{e}	5.26 ^{de}
T_4	11.61 ^{a}	52.65 ^{e}	2.48 ^{f}	5.55 ^{b}
T_5	11.63 ^{a}	52.36 ^{fg}	2.22 ^{h}	5.32 ^{cd}
T_6	11.65 ^{a}	52.21 ^{g}	2.11 ^{j}	5.24 ^{e}
T_7	11.68 ^{a}	54.80 ^{b}	3.06 ^{a}	5.18 ^{e}
T_8	11.66 ^{a}	54.51 ^{d}	2.98 ^{b}	5.08 ^{f}
T_9	11.65 ^{a}	54.62 ^{cd}	2.95 ^{c}	4.85 ^{gh}
T_{10}	11.65 ^{a}	52.45 ^{f}	2.44 ^{g}	4.92 ^{g}
T_{11}	11.62 ^{a}	51.93 ^{h}	2.15 ^{i}	4.98 ^{fg}
T_{12}	11.64 ^{a}	52.28 ^{fg}	2.14 ^{i}	4.76 ^{h}
T_{13}	11.68 ^{a}	54.59 ^{d}	2.87 ^{d}	5.02 ^{f}
T_{14}	11.67 ^{a}	52.36 ^{fg}	2.21 ^{h}	4.83 ^{h}

Values are mean of three independent determinations
Values with same superscript do not have significant difference
DMRT column wise comparison

Table 18. TSS (°Brix), starch, reducing sugar and total sugars in unfermented food mixtures (g/100g)

Treatments	TSS	Starch	Reducing Sugar	Total Sugars
T_1	14.863 ^{b}	65.730 ^{c}	4.767 ^{b}	9.967 ^{a}
T_2	14.973 ^{a}	66.943 ^{a}	4.677 ^{d}	9.740 ^{c}
T_3	14.920 ^{d}	65.553 ^{e}	4.230 ^{f}	9.560 ^{d}
T_4	14.883 ^{f}	62.310 ^{h}	3.433 ^{g}	9.517 ^{e}
T_5	14.953 ^{b}	62.140 ^{i}	3.343 ^{h}	9.440 ^{f}
T_6	14.930 ^{c}	61.967 ^{k}	3.063 ^{k}	9.263 ^{h}
T_7	14.860 ^{h}	65.933 ^{b}	4.963 ^{a}	9.567 ^{d}
T_8	14.930 ^{c}	65.423 ^{f}	4.720 ^{c}	9.353 ^{g}
T_9	14.923 ^{d}	65.543 ^{e}	4.733 ^{c}	9.023 ^{j}
T_{10}	14.863 ^{h}	62.230 ^{g}	3.440 ^{g}	9.143 ^{i}
T_{11}	14.890 ^{f}	61.673 ^{c}	3.240 ^{i}	9.043 ^{j}
T_{12}	14.870 ^{g}	62.073 ^{j}	3.170 ^{j}	9.357 ^{g}
T_{13}	14.937 ^{c}	65.620 ^{d}	4.577 ^{e}	9.813 ^{b}
T_{14}	14.910 ^{e}	61.337 ^{m}	3.247 ^{i}	9.250 ^{h}

Values are mean of three independent determinations
Values with same superscript do not have significant difference
DMRT column wise comparison

FFM and UFFM were statistically compared for their TSS, starch, reducing sugar and total sugar by applying independent sample 't' test and is presented in Table 19.

Table 19. TSS, starch, reducing sugar and total sugars in FFM and UFFM.

Methods	TSS(g)	Starch(g)	Reducing sugar(g)	Total sugars(g)
FFM	11.65	53.51	2.60	5.14
UFFM	14.91	63.89	3.97	9.43
Mean difference	-3.258	-10.38	-1.371	-4.29
t value	-489.69	-28.72	-10.89	-1.186
Significance	.0001	.0001	.0001	.0001
	S	S	S	S

From the table it was revealed that there was a significant difference ($p<0.05$) in the case of TSS, starch, reducing sugar and total sugars in FFM when compared to UFFM. All the above constituents were significantly low in FFM. The TSS, starch, reducing sugar and total sugars are depicted in Fig 13-16.

Table 20. Calcium, potassium and iron in fermented food mixtures (mg/100g)

Treatments	Calcium	Potassium	Iron
T_1	67.77 ^{j}	305.33 ^{h}	6.99 ^{a}
T_2	67.31 ^{de}	304.67 ^{i}	6.33 ^{e}
T_3	69.70 ^{a}	396.67 ^{d}	6.79 ^{c}
T_4	43.92 ^{i}	483.00 ^{j}	6.32 ^{e}
T_5	44.71 ^{g}	468.00 ^{k}	6.04 ^{h}
T_6	46.90 ^{f}	492.67 ^{a}	6.13 ^{g}
T_7	68.25 ^{b}	307.33 ^{g}	6.13 ^{g}
T_8	66.94 ^{e}	313.67 ^{f}	6.26 ^{f}
T_9	69.12 ^{a}	306.00 ^{e}	6.24 ^{f}
T_{10}	43.82 ^{j}	492.67 ^{a}	6.72 ^{a}
T_{11}	44.22 ^{h}	486.00 ^{b}	6.85 ^{b}
T_{12}	43.82 ^{j}	482.00 ^{c}	6.66 ^{d}
T_{13}	67.41 ^{cd}	317.00 ^{e}	6.97 ^{a}
T_{14}	45.00 ^{g}	487.00 ^{b}	6.28 ^{f}

Values are mean of three independent determinations
Values with same superscript do not have significant difference
DMRT column wise comparison

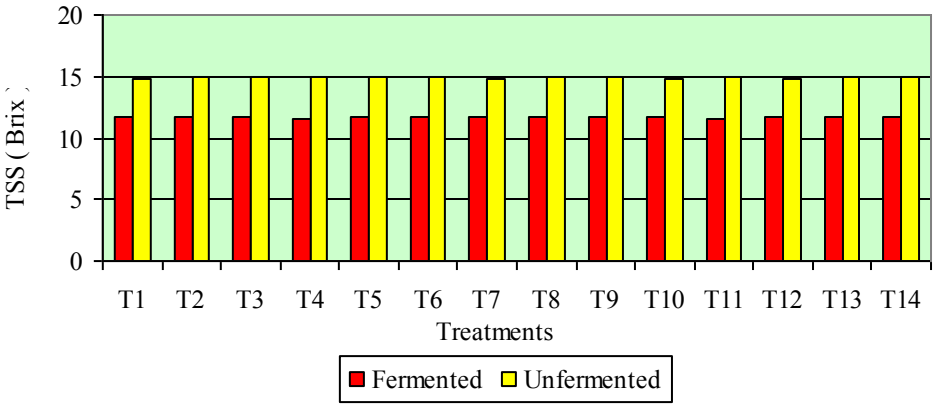


Fig. 13. TSS in fermented and unfermented food mixtures

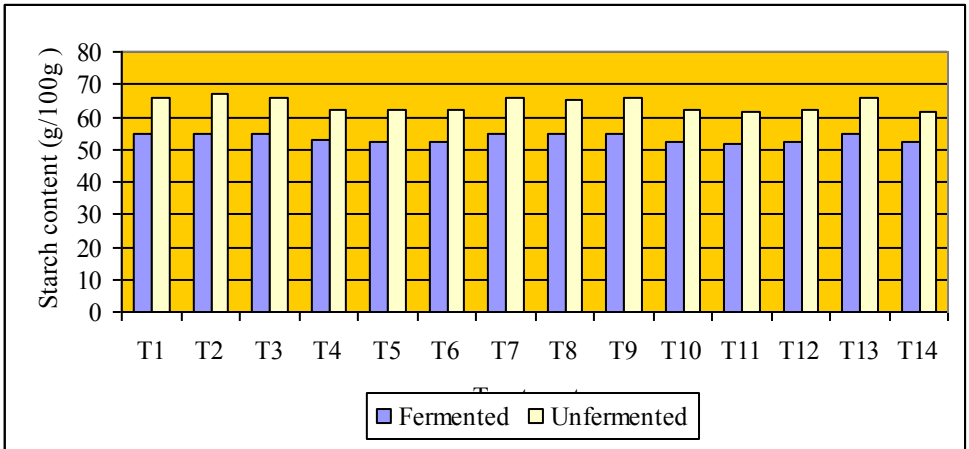


Fig. 14. Starch content in fermented and unfermented food mixtures

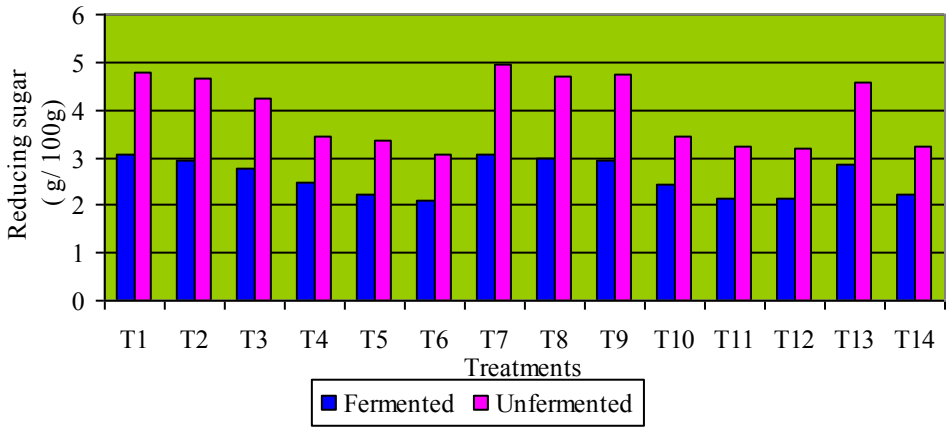


Fig. 15. Reducing Sugar in fermented and unfermented food mixtures

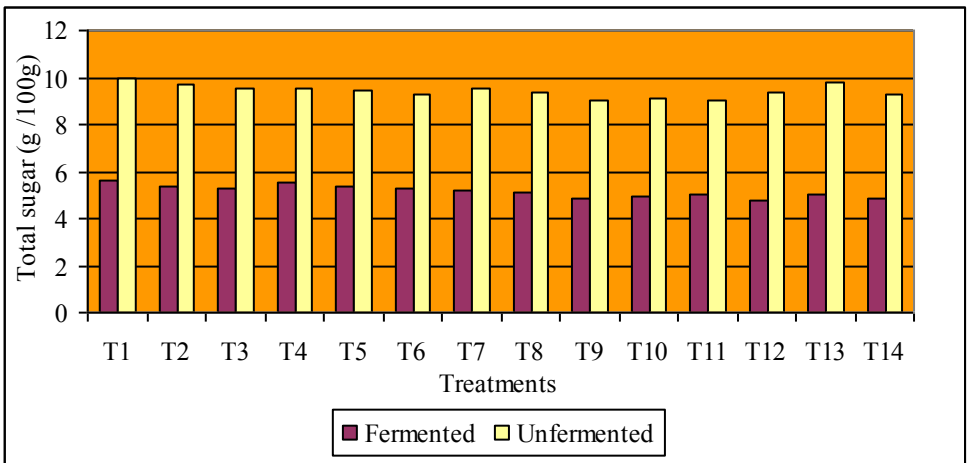


Fig. 16. Total Sugar in fermented and unfermented food mixtures

As revealed in Table 20, there was a significant difference in the calcium content of FFM. T3 had the highest calcium content of 69.70 mg/100g whereas T_{10} and T_{12} showed the least calcium content of 43.82 mg/100g. High potassium content was observed in all FFM which varied from 304.67 mg in T_2 to 492.67 mg/100g in T_6 and T10 and the difference in the potassium content observed in FFM were significant. Iron content of FFM ranged from 6.04mg in T_5 to 6.99mg/ 100 g in T_1 and the difference in iron content was also found to be significant

Table 21. Calcium, potassium and iron in unfermented food mixtures (mg/100g)

Treatments	Calcium	Potassium	Iron
T_1	67.693 ^{d}	304.333 ^{h}	6.90 ^{a}
T_2	67.283 ^{e}	308.333 ^{g}	6.32 ^{d}
T_3	69.233 ^{b}	393.333 ^{e}	6.77 ^{b}
T_4	43.697 ^{j}	484.667 ^{b}	6.23 ^{e}
T_5	44.640 ^{g}	468.000 ^{d}	6.04 ^{g}
T_6	46.570 ^{a}	497.333 ^{a}	6.15 ^{f}
T_7	67.400 ^{e}	307.000 ^{a}	6.17 ^{f}
T_8	66.550 ^{a}	313.000 ^{f}	6.26 ^{e}
T_9	68.843 ^{a}	312.667 ^{f}	6.21 ^{e}
T_{10}	44.277 ^{h}	495.000 ^{a}	6.73 ^{a}
T_{11}	43.887 ^{i}	486.333 ^{b}	6.74 ^{b}
T_{12}	43.360 ^{k}	481.333 ^{c}	6.62 ^{c}
T_{13}	66.817 ^{a}	316.000 ^{e}	6.85 ^{a}
T_{14}	45.037 ^{a}	487.000 ^{b}	6.23 ^{e}

Values are mean of three independent determinations
Values with same superscript do not have significant difference
DMRT column wise comparison

There was a significant difference in the calcium content of UFFM which ranged between 43.36 mg/100g in T_{12} and 69.23mg/100g in T_3. Potassium content ranged between 304.33mg/100g in T_1 and 497.33mg/100g in T_6 and the difference was significant. Iron content also showed a significant difference which ranged from 6.04mg/100g in T_5 to 6.90 mg/100g in T_1. Thus a significant variation was observed in the mineral content of UFFM.

FFM and UFFM were statistically compared for their calcium, potassium and iron by applying independent sample‘t’ test and is presented in Table 22.

Table 22. Calcium, potassium and iron in fermented and unfermented food mixtures

Methods	Calcium(mg)	Potassium(mg)	Iron(mg)
FFM	56.35	403.00	6.481
UFFM	56.09	403.81	6.445
Mean difference	0.257	-0.809	0.036
t value	0.100	-0.043	1.957
Significance	0.921	0.966	.0001
	NS	NS	S

There was no significant variation in calcium and potassium content of FFM and UFFM but iron was found to be significantly high in FFM. The calcium, potassium and iron in FFM and UFFM is shown in Fig 17-19.

Table 23. Thiamine and riboflavin content in fermented food mixtures (mg/100g)

Treatments	Thiamine	Riboflavin
T_1	0.085 ^{b}	0.65 ^{a}
T_2	0.073 ^{d}	0.61 ^{c}
T_3	0.089 ^{a}	0.67 ^{a}
T_4	0.066 ^{fg}	0.45 ^{d}
T_5	0.063 ^{h}	0.43 ^{e}
T_6	0.069 ^{e}	0.46 ^{d}
T_7	0.083 ^{b}	0.62 ^{bc}
T_8	0.085 ^{b}	0.64 ^{b}
T_9	0.075 ^{c}	0.61 ^{e}
T_{10}	0.064 ^{g}	0.40 ^{f}
T_{11}	0.066 ^{f}	0.45 ^{d}
T_{12}	0.061 ^{h}	0.43 ^{e}
T_{13}	0.073 ^{d}	0.62 ^{bc}
T_{14}	0.064 ^{gh}	0.45 ^{d}

Values are mean of three independent determinations
Values with same superscript do not have significant difference
DMRT column wise comparison

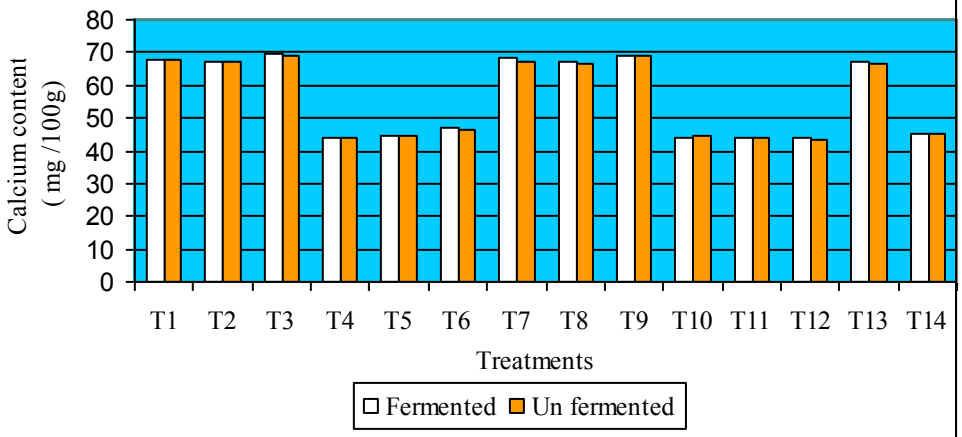


Fig.17. Calcium content in fermented and unfermented food mixtures

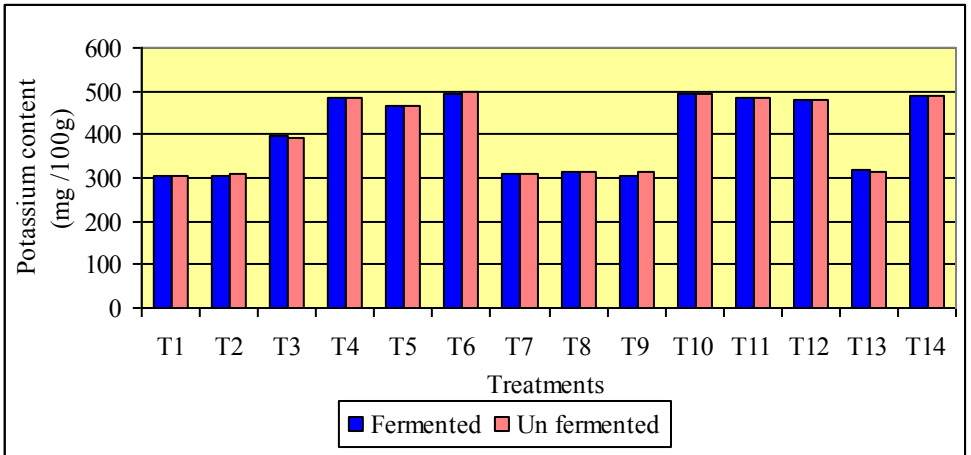


Fig. 18. Potassium content in fermented and unfermented food mixtures

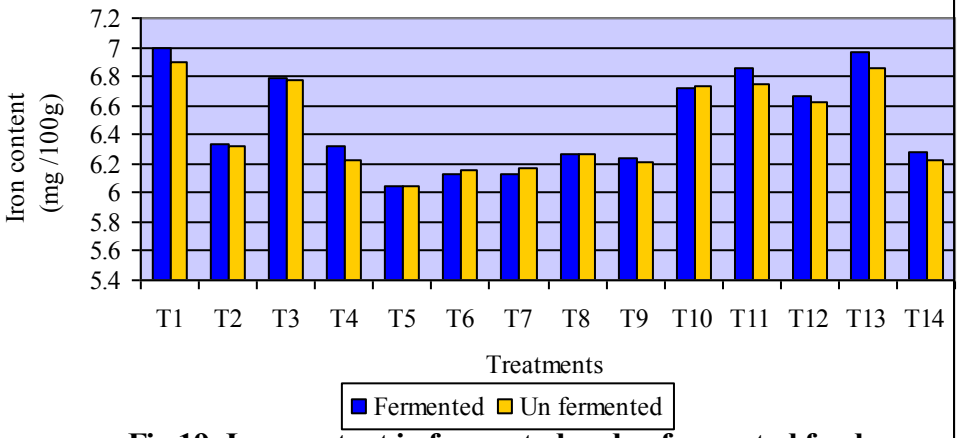


Fig.19. Iron content in fermented and unfermented food mixtures

As shown in Table 23, thiamine content ranged between 0.061 to 0.089 mg/100g and riboflavin content ranged between 0.40 to 0.67 mg/100gm in the fermented food mixtures and there was a significant difference between the treatments in both thiamine and riboflavin. Thiamine and riboflavin were significantly high in T_3

Table 24. Thiamine and riboflavin content in unfermented food mixtures (mg/100g)

Treatments	Thiamine	Riboflavin
T_1	0.066 ^{ab}	0.35 ^{ab}
T_2	0.053 ^{d}	0.31 ^{c}
T_3	0.056 ^{d}	0.32 ^{c}
T_4	0.045 ^{f}	0.27 ^{d}
T_5	0.045 ^{fg}	0.23 ^{ef}
T_6	0.047 ^{e}	0.25 ^{de}
T_7	0.065 ^{bc}	0.34 ^{ab}
T_8	0.068 ^{a}	0.36 ^{a}
T_9	0.055 ^{de}	0.33 ^{bc}
T_{10}	0.045 ^{fg}	0.23 ^{f}
T_{11}	0.047 ^{ef}	0.25 ^{de}
T_{12}	0.043 ^{h}	0.22 ^{f}
T_{13}	0.063 ^{c}	0.33 ^{bc}
T_{14}	0.067 ^{a}	0.24 ^{e}

Values are mean of three independent determinations
Values with same superscript do not have significant difference
DMRT column wise comparison

As shown in Table 24, thiamine content ranged between 0.043 to 0.068 mg/100g and riboflavin content ranged between 0.22 to 0.36 mg/100gm in UFFM and there was a significant difference in thiamine and riboflavin between the treatments.

FFM and UFFM were statistically compared for their thiamine and riboflavin content by applying independent sample‘t’ test and is presented in table 25.

Table 25. Thiamine and riboflavin in FFM and UFFM.

Methods	Thiamine(mg)	Riboflavin(mg)
FFM	0.0726	0.535
UFFM	0.0540	0.284
Mean difference	0.0182	0.251
T value	5.081	8.235
Significance	.0001	.0001
	S	S

Table 25 revealed that there was a significant increase in the case of thiamine and riboflavin in FFM was significantly high when compared to the UFFM. The thiamine and riboflavin content of FFM and UFFM is given in Fig 20 and 21.

4.5.2. IVSD in fermented and unfermented food mixtures

Table 26 shows the IVSD in FFM and UFFM.

Table 26. IVSD in fermented and unfermented food mixtures

Treatments	IVSD (%)	
	FFM	UFFM
T_1	80.17 ^{g}	56.17 ^{f}
T_2	78.57 ^{h}	54.46 ^{g}
T_3	80.50 ^{g}	56.02 ^{e}
T_4	83.60 ^{a}	56.21 ^{b}
T_5	82.93 ^{cd}	56.23 ^{b}
T_6	83.07 ^{bc}	56.17 ^{c}
T_7	81.50 ^{f}	54.46 ^{g}
T_8	82.47 ^{e}	56.05 ^{e}
T_9	82.73 ^{cde}	56.34 ^{a}
T_{10}	83.40 ^{ab}	54.32 ^{j}
T_{11}	83.37 ^{ab}	54.36 i
T_{12}	82.90 ^{cd}	54.41 ^{h}
T_{13}	81.73 ^{f}	56.11 ^{d}
T_{14}	82.60 ^{de}	54.43 ^{gh}

Values are mean of three independent determinations
Values with same superscript do not have significant difference
DMRT column wise comparison

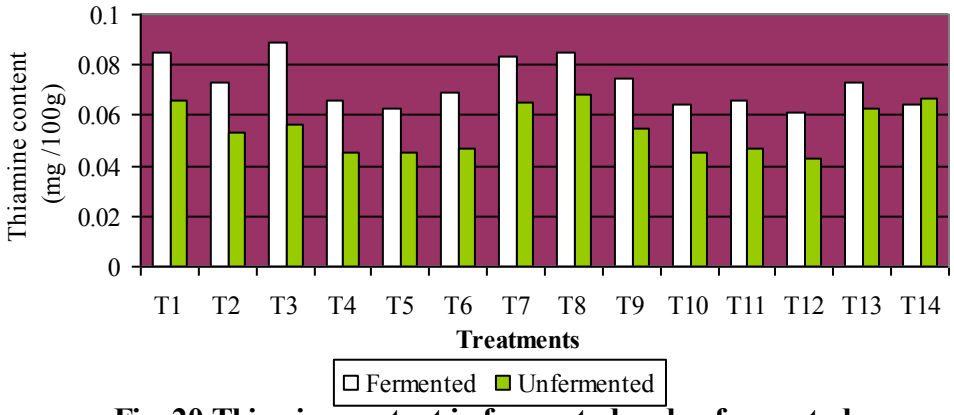


Fig. 20.Thiamine content in fermented and unfermented food mixtures

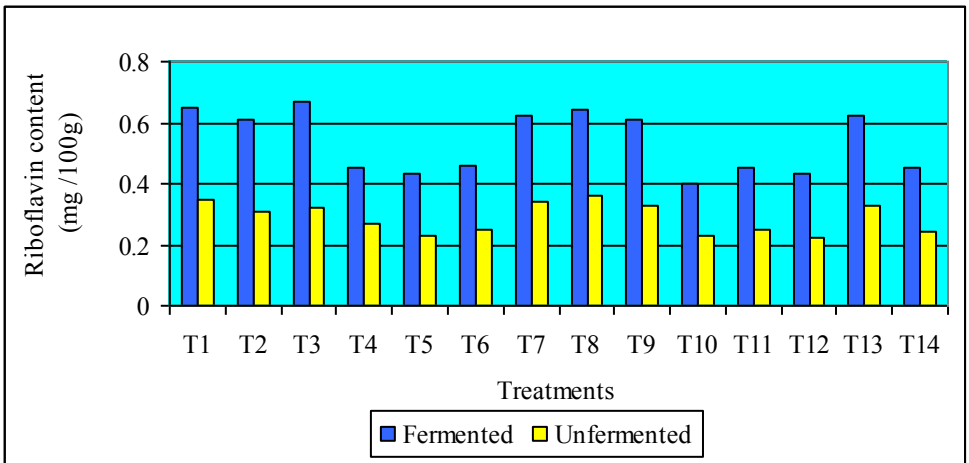


Fig.21.Riboflavin content in fermented and unfermented food mixtures

Among FFM, IVSD was maximum in T_4 (83.60 per cent) and minimum in T_2 (78.57 per cent). There was no significant difference in IVSD of T_4 with that of T_{10} (83.4 per cent) and T_{11} (83.37 per cent). Among UFFM, maximum IVSD was in T_9 (56.34 per cent) and least IVSD was in T_{12} (54.41 per cent).

4.5.3. IVPD in fermented and unfermented food mixtures

Table 27 shows the IVPD in FFM and UFFM.

Table 27. IVPD in fermented and unfermented food mixtures

Treatments	IVPD (%)	
	FFM	UFFM
T_1	85.14 ^{g}	57.15 ^{h}
T_2	85.83 ^{d}	57.78 ^{c}
T_3	85.56 ^{f}	57.23 ^{g}
T_4	86.21 ^{a}	57.82 ^{b}
T_5	86.11 ^{c}	57.65 ^{d}
T_6	86.15 ^{b}	57.87 ^{a}
T_7	85.74 ^{e}	57.56 ^{e}
T_8	85.15 ^{g}	57.15 ^{h}
T_9	85.55 ^{f}	57.45 ^{f}
T_{10}	86.18 ^{ab}	57.65 ^{c}
T_{11}	86.19 ^{ab}	57.61 ^{c}
T_{12}	86.16 ^{b}	57.64 ^{c}
T_{13}	85.74 ^{e}	57.42 ^{f}
T_{14}	86.17 ^{b}	57.67 ^{d}

Values are mean of three independent determinations
Values with same superscript do not have significant difference
DMRT column wise comparison

There was a significant variation in the IVPD of FFM and UFFM. IVPD of FFM varied from 85.14 to 86.21 per cent, the maximum in T_4. T_4 showed no significant variation with T_{10} (86.18 per cent) and T_{11} (86.19 per cent).Among UFFM, maximum IVPD was in T_6 (57.87 per cent) and the least in T_1 and T_8 (57.15 per cent)

FFM and UFFM were statistically compared for their IVSD and IVPD by applying independent sample‘t’ test and is presented in Table 28.

Table 28. IVSD and IVPD in FFM and UFFM

Methods	IVSD (per cent)	IVPD(per cent)
FFM	82.109	85.850
UFFM	55.340	57.540
Mean difference	26.76	28.30
t value	103.49	416.57
Significance	.0001	.0001
	S	S

Table 28 revealed that IVSD and IVPD in FFM were significantly high when compared to UFFM. The IVSD and IVPD of FFM and UFFM is depicted in Fig 22 and 23.

4.5.4. Viable count of *L. acidophilus* in fermented and unfermented food mixtures

L. acidophilus present in FFM were enumerated and the results are presented in table 29.

Table 29. Viable cell count of *L. acidophilus* in fermented food mixtures

Treatment	Viable count	
	(x 10^7 cfu/g)	log cfu /g
UFFM	Nil	Nil
FFM		
T_1	147	9.167
T_2	139	9.143
T_3	282	9.451
T_4	159	9.201
T_5	143	9.155
T_6	292	9.465
T_7	137	9.136
T_8	210	9.322
T_9	205	9.311
T_{10}	147	9.167
T_{11}	188	9.276
T_{12}	175	9.245
T_{13}	136	9.136
T_{14}	275	9.439

Values are mean of 3 independent enumerations

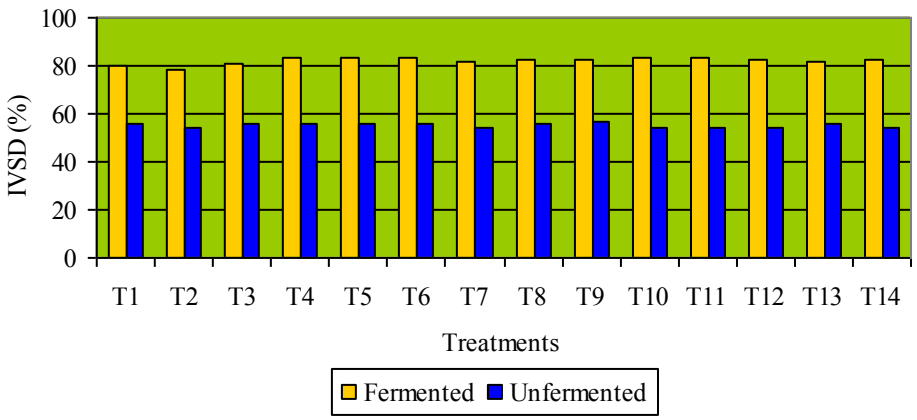


Fig. 22. IVSD of fermented and unfermented food mixtures

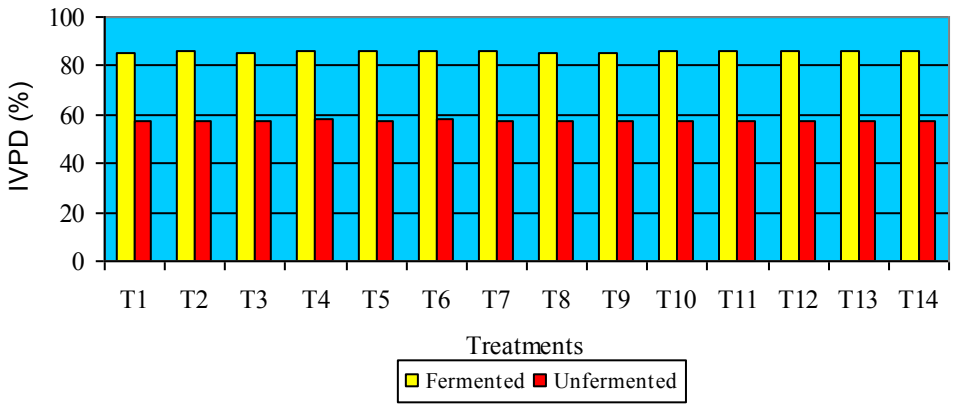


Fig. 23. IVPD of fermented and unfermented food mixtures

As revealed in Table 29, maximum viable count was observed in T_6 (292 x 10^7 cfu /g) and the least in T_{13} (136 x 10^7cfu /g). As expressed in log cfu/g, the viable count of *L. acidophilus* in the treatments varied from 9.13 to 9.46 log cfu/g (Fig 24) as against the desired level of 4.7 to 8.9 log cfu/g in probiotic foods.

4.5.5. Organoleptic qualities of the food mixtures

Probiotic fermented food mixtures (5gm) mixed with 100ml of diluted buttermilk (1:4) were subjected to sensory evaluation .The corresponding control of unfermented samples were also presented in the same way (Plate 9). Mean scores obtained for both FFM and UFFM for different quality criteria were calculated and presented in Table 30.

Table 30. Mean score for organoleptic qualities of food mixtures fermented with *L. acidophilus*

Treatments	Mean score					
	Appearance	Colour	Flavour	Texture	Taste	Overall Acceptability
T_1	8.3	8.5	7.1	8.2	7.3	7.9
T_2	8.4	8.4	7.3	8.4	7.3	8.0
T_3	8.3	8.6	7.1	8.3	7.5	7.9
T_4	8.3	8.4	7.0	8.3	7.7	7.9
T_5	8.2	8.5	6.8	8.3	7.6	7.9
T_6	8.3	8.5	7.2	8.3	7.8	8.0
T_7	8.2	8.6	7.0	8.4	7.3	7.9
T_8	8.4	8.4	7.0	8.3	7.4	7.9
T_9	8.4	8.5	7.2	8.3	7.4	7.9
T_{10}	8.2	8.5	7.2	8.2	7.5	7.9
T_{11}	8.4	8.5	7.0	8.3	7.7	8.0
T_{12}	8.2	8.6	7.1	8.2	7.6	7.9
T_{13}	8.3	8.5	7.3	8.2	7.4	7.9
T_{14}	8.3	8.5	7.3	8.2	7.7	8.0

As revealed in the table, the mean score for appearance, colour and texture of the fermented samples were liked very much (between 8-9 in hedonic scale) where as

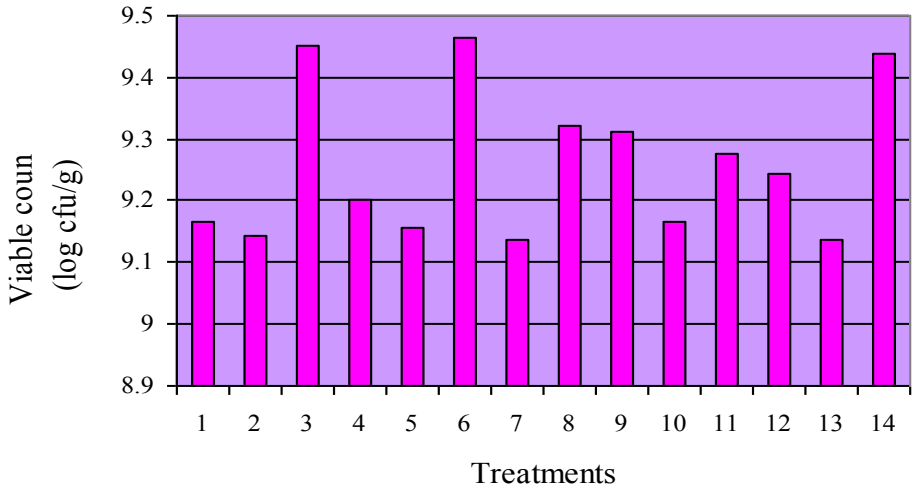


Fig. 24.Viable count of *L.acidophilus* in the food mixtures

Plate 9. Presentation of samples for organoleptic evaluation

flavour, taste and over all acceptability were moderately liked (between 7-8 hedonic scale) by the panelists.

Table 31. Mean score for organoleptic qualities of unfermented food mixture

Treatments	Mean score					
	Appearance	Colour	Flavour	Texture	Taste	Overall Acceptability
T_1	8.3	8.4	5.6	8.3	5.5	7.2
T_2	8.3	8.4	5.8	8.2	5.6	7.3
T_3	8.2	8.5	5.8	8.2	5.7	7.3
T_4	8.2	8.6	5.7	8.2	5.7	7.3
T_5	8.3	8.5	5.6	8.2	5.7	7.3
T_6	8.3	8.5	5.6	8.2	5.6	7.2
T_7	8.3	8.6	5.8	8.2	5.6	7.3
T_8	8.4	8.4	5.5	8.4	5.8	7.3
T_9	8.3	8.5	5.8	8.2	5.5	7.3
T_{10}	8.3	8.4	5.8	8.3	5.8	7.3
T_{11}	8.4	8.4	5.5	8.3	5.8	7.3
T_{12}	8.3	8.6	5.6	8.3	5.6	7.3
T_{13}	8.3	8.5	5.7	8.4	5.8	7.3
T_{14}	8.4	8.5	5.7	8.3	5.8	7.3

As shown in Table 31, the mean score for appearance, colour and texture of UFFM were liked very much (between 8-9 in hedonic scale)whereas flavour and taste were neither liked nor disliked (between 5and 6 in hedonic scale) by the panelists.

Table 32. Comparison of organoleptic qualities of fermented and unfermented food mixtures

Methods	Appearance	Colour	Flavour	Texture	Taste	OAA
Fermented	8.300	8.486	7.110	8.271	7.510	7.825
Unfermented	8.307	8.485	5.680	8.271	5.680	7.690
Mean difference	0.007	0.001	1.430	0.000	1.830	0.135
t value	0.041	0.044	21.35	0.039	26.34	7.154
Significance	NS	NS	S	NS	S	S

Values are mean of ten panelists

Statistical analysis by applying independent t test it was revealed that (Table 32) there was no significant difference between the appearance, colour and texture of fermented and unfermented food mixtures but fermented mixtures had significantly

($p<0.05$) higher acceptability scores with regard to flavour, taste and overall acceptability (Fig 25)

4.6. Selection of six food mixtures with maximum quality attributes

Based on the nutrient composition, acceptability and presence of viable count of *L. acidophilus* in the fourteen FFM, six FFM with maximum quality attributes were selected by applying geometric mean scores and is presented in Table 33.

Table 33. Six fermented food mixtures with maximum quality attributes

Treatment with composition		Geometric mean scores
T_1	B+ DS+M	**23.673**
T_2	B+ DS+P	**22.868**
T_3	B+ DS+T	**25.101**
T_4	B+ GG+M	21.708
T_5	B+ GG+P	20.700
T_6	B+ GG+T	22.099
T_7	B+ DS+M+P	**23.009**
T_8	B+ DS+M+T	**23.758**
T_9	B+ DS+P+T	**23.354**
T_{10}	B+ GG+M+P	21.183
T_{11}	B+ GG+M+T	20.811
T_{12}	B+ GG+P+T	20.769
T_{13}	B+ DS+M+P+T	22.824
T_{14}	B+ GG+M+P+T	22.043

Highest geometric mean score was for T_3 (25.101) followed by T_8 (23.758), T_1 (23.673), T_9 (23.354), T_7 (23.09) and T_2 (22.868).These six food mixtures along with their control was selected for further studies. In all the selected six food mixtures, defatted soya was used as a protein source.

4.7. Storage Studies of the selected six food mixtures

The selected six food mixtures (along with unfermented controls) were packed in metalised polyester /polyethylene laminated pouches (Plate 10) and were stored for 6 months under ambient conditions, and the results of the quality evaluation conducted

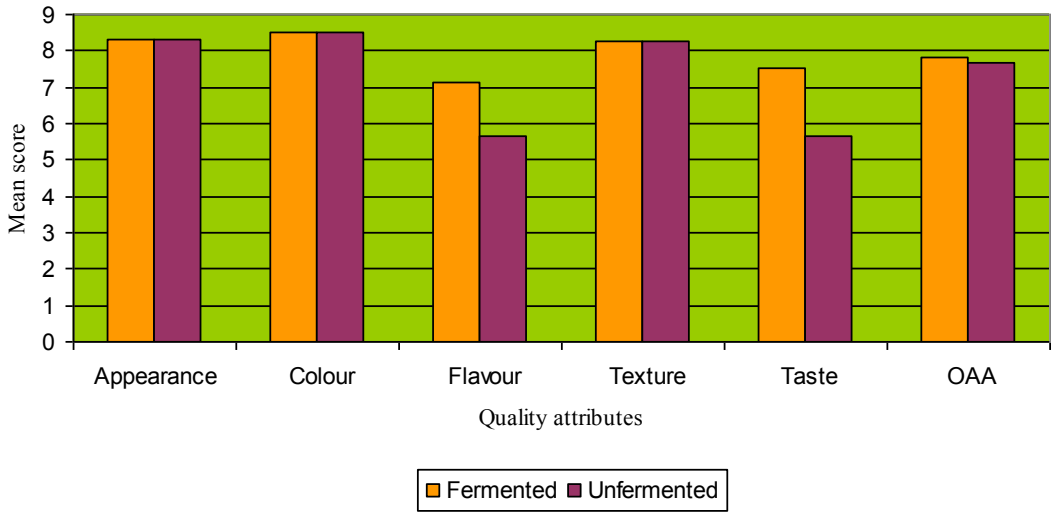


Fig. 25. Organoleptic qualities of fermented and unfermented food mixtures

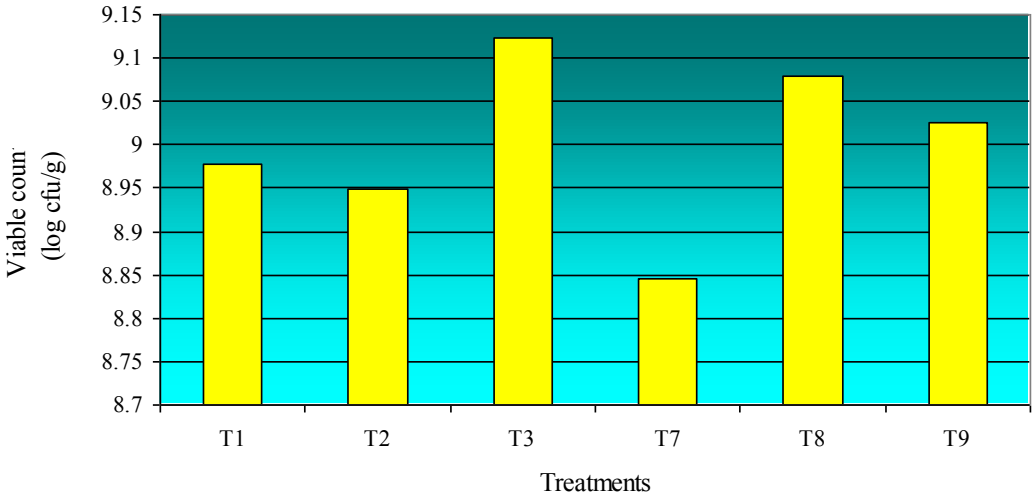


Fig. 26. Viable count of *L.acidophilus* in FFM after six months of storage

Plate 10. Packing of food mixtures in metalised polyester/polyethylene laminated pouches

for each month is presented in the following tables. Statistical significance was found out using Duncan's multiple range test and is also presented in the tables.

4.7.1. Chemical constituents

4.7.1.1 Moisture

Table 34 .Moisture content of fermented food mixtures on storage (g/100g)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	1.803 [a]	1.803 [a]	1.867 [b]	2.133 [c]	2.233 [d]	2.523 [e]	2.970 [f]
T_2	1.847 [a]	1.857 [a]	1.927 [b]	2.187 [c]	2.260 [d]	2.593 [e]	3.027 [f]
T_3	1.837 [a]	1.843 [a]	1.903 [b]	2.140 [c]	2.243 [d]	2.547 [e]	2.980 [f]
T_7	1.847 [a]	1.850 [a]	1.947 [b]	2.183 [c]	2.283 [d]	2.603 [e]	3.017 [f]
T_8	1.845 [a]	1.847 [a]	1.943 [b]	2.167 [c]	2.253 [d]	2.587 [e]	3.013 [f]
T_9	1.840 [a]	1.843 [a]	1.907 [b]	2.143 [c]	2.233 [d]	2.337 [e]	2.976 [f]

Values having different super script differ significantly at 5% level
DMRT Row wise comparison

Moisture content showed no significant difference upto one month of storage in all the treatments but after that increased significantly throughout the storage period. Maximum moisture content was in T_7 (1.947g/100 g) and minimum in T_1 (1.867/100g) during the second month. There was a significant increase during the third month in all the treatments, with maximum in T_2 (2.187g/100g) and minimum in T_1 (2.133g/100gm). T_7 showed maximum moisture content in the fourth and fifth month (2.283 and 2.603g/100g respectively) but by the end of sixth month, T_2 showed the maximum moisture content of 3.027 g/100g. T_1 maintained its least moisture content in fourth month (2.233g/100g) but during the fifth month, least moisture content was observed in T_9 (2.337g/100g). By the end of the storage period, least moisture content was again observed in T_1 (2.970g/100g). Thus, in the selected six FFM, the mean moisture content of 1.837 g/100g initially, was increased to a mean of 1.840 g during the second month which showed no significant difference but by the sixth month, the mean moisture content was significantly increased to 2.997 g/100g with a maximum moisture content in T_2 with 3.027g/100g and the least in T_1 with 2.970g/100g.

Table 35.Moisture content of unfermented food mixtures on storage (g/100g)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	1.803 [a]	1.807 [a]	1.857 [b]	2.117 [c]	2.213 [d]	2.513 [e]	2.960 [f]
T_2	1.837 [a]	1.843 [a]	1.896 [b]	2.183 [c]	2.263 [d]	2.603 [e]	3.013 [f]
T_3	1.843 [a]	1.853 [a]	1.897 [b]	2.147 [c]	2.173 [d]	2.540 [e]	2.980 [f]
T_7	1.833 [a]	1.837 [a]	1.970 [b]	2.167 [c]	2.267 [d]	2.597 [e]	3.020 [f]
T_8	1.833 [a]	1.833 [a]	1.953 [b]	2.173 [c]	2.277 [d]	2.607 [e]	3.016 [f]
T_9	1.817 [a]	1.820 [a]	1.913 [b]	2.143 [c]	2.247 [d]	2.540 [e]	2.973 [f]

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Moisture content in UFFM showed no significant difference upto one month of storage in all the treatments but increased significantly throughout the storage period. Maximum moisture content was in T_7 (1.970 g/100 g) and minimum in T_1 (1.857g /100g) during the second month. There was a significant increase during the third month in all the treatments, with maximum in T_2 (2.183g/100g) and minimum in T_1 (2.117g/100g). T_8 showed maximum moisture content in the fourth and fifth month (2.277 and 2.607g/100g respectively) but by the end of sixth month, T_7 showed the maximum moisture content of 3.020 g/100g. T_1 maintained its least moisture content in fourth , fifth and sixth month (2.213, 2.513 and 2.960 g/100g respectively) .The mean moisture content of 1.828 g/100g in UFFM initially, was increased to a mean of 1.831g/100g during the first month, and reached to a mean value of 2.994 g/100g towards the end of storage study.

The moisture content of FFM and UFFM on storage, was statistically compared by applying independent sample‘t’ test and is presented in Table 36.

Table 36 Moisture content of fermented and unfermented food mixtures on storage (g/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	1.837	1.839	1.916	2.158	2.251	2.565	2.997
UFFM	1.828	1.831	1.914	2.155	2.250	2.567	2.994
Mean difference	.0083	.0083	.0011	.0039	.0011	-.0017	.0033
T value	1.542	1.467	0.097	0.498	0.132	-0.140	0.406
Significance	0.132	0.152	0.923	0.621	0.896	0.889	0.687
	NS	NS	NS	NS	NS	NS	NS

In both FFM and UFFM, the moisture content increased significantly on storage, but there was no significant variation in the moisture content of FFM and UFFM during storage.

4.7.1.2 Titrable acidity

Table 37.Titrable acidity of fermented food mixtures on storage (g lactic acid/100g)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	2.467 ^{a}	2.547 ^{b}	2.570 ^{c}	2.713 ^{d}	2.840 ^{e}	3.170 ^{f}	3.277 ^{g}
T_2	2.450 ^{a}	2.557 ^{b}	2.563 ^{c}	2.750 ^{d}	2.867 ^{e}	3.217 ^{f}	3.320 ^{g}
T_3	2.537 ^{a}	2.607 ^{b}	2.653 ^{c}	2.773 ^{d}	3.027 ^{e}	3.250 ^{f}	3.367 ^{g}
T_7	2.540 ^{a}	2.603 ^{b}	2.640 ^{c}	2.787 ^{d}	3.020 ^{e}	3.257 ^{f}	3.377 ^{g}
T_8	2.540 ^{a}	2.617 ^{b}	2.653 ^{c}	2.763 ^{d}	2.870 ^{e}	3.227 ^{f}	3.320 ^{g}
T_9	2.536 ^{a}	2.577 ^{b}	2.616 ^{c}	2.723 ^{d}	2.833 ^{e}	3.170 ^{f}	3.277 ^{g}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Titrable acidity was significantly increasing with the storage period in all the treatments. Initially maximum titrable acidity was in T_7 and T_8 (2.540g/100 g) and minimum in T_2 (2.450g/100g). During the first month, there was a significant increase in all the treatments with maximum in T_8 (2.617g/100g) and minimum in T_1 (2.547g/100g). T_3 and T_8 showed maximum titrable acidity in the second month

(2.653g/100g). During the third and fifth month T_7 showed the maximum titrable acidity (2.787 and 3.257g/100g respectively) and the least was in T_1 and T_9 (2.713 and 3.17 g/100g respectively). T_7 showed the maximum titrable acidity in the fifth and sixth month (3.257 and 3.337g/100g respectively). The least titrable acidity was in T_1 and T_9 (3.170 g/100g) by the end of the sixth month. Thus the mean titrable acidity of 2.509 g/100g in FFM during the initial period was significantly increased to a mean value of 3.238 g/100g towards the end of storage study.

Table 38. Titrable acidity of unfermented food mixtures on storage (g lactic acid/100g)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	1.250 ^{a}	1.257 ^{a}	1.303 ^{b}	1.373 ^{c}	1.487 ^{d}	1.660 ^{e}	1.967 ^{f}
T_2	1.267 ^{a}	1.273 ^{a}	1.317 ^{b}	1.457 ^{c}	1.533 ^{d}	1.717 ^{e}	2.020 ^{f}
T_3	1.240 ^{a}	1.247 ^{a}	1.310 ^{b}	1.417 ^{c}	1.537 ^{d}	1.740 ^{e}	2.033 ^{f}
T_7	1.273 ^{a}	1.277 ^{a}	1.337 ^{b}	1.443 ^{c}	1.533 ^{d}	1.777 ^{e}	2.060 ^{f}
T_8	1.237 ^{a}	1.240 ^{a}	1.270 ^{b}	1.347 ^{c}	1.440 ^{d}	1.670 ^{e}	1.963 ^{f}
T_9	1.230 ^{a}	1.234 ^{a}	1.277 ^{b}	1.367 ^{c}	1.443 ^{d}	1.650 ^{e}	1.970 ^{f}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Titrable acidity was significantly increasing with the storage period in all the treatments. Initially maximum acidity was in T_7 (1.273g/100 g) and minimum in T_9 (1.230g/100g). During the first month there was no significant increase in any of the treatments. T_7 showed a maximum significant increase in the second month (1.337g/100g) and minimum in T_8 (1.270 g/100g). T_2 showed a maximum increase in titrable acidity in the third month (1.347g/100g) and the least was in T_8 (1.347 g/100g). In the fourth month, T_3 (1.537g/100g) showed maximum titrable acidity and T_8 showed minimum acidity of 1.440g/100g. T_7 showed the maximum titrable acidity of 1.777 g/100g and 2.060 g/100g during the fifth and sixth month whereas minimum titrable acidity was in T_9 (1.650 g/100g) and T_8 (1.963g/100g) during the fifth and sixth month respectively.

Thus in UFFM, the mean initial titrable acidity of 1.255g/100g was significantly increased to a mean value of 2.002 g/100g towards the sixth month.

The titrable acidity of FFM and UFFM were statistically compared by applying independent sample't' test and is presented in Table 39.

Table 39. Titrable acidity of fermented and unfermented food mixtures on storage (g lactic acid/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	2.509	2.584	2.616	2.751	2.909	3.215	3.328
UFFM	1.255	1.252	1.302	1.401	1.496	1.702	2.002
Mean difference	1.254	1.332	1.313	1.351	1.413	1.513	1.321
t value	97.22	169.61	121.91	113.00	63.28	105.63	98.26
Significance ($p<0.05$)	S	S	S	S	S	S	S

In FFM, the titrable acidity was significantly high throughout the storage period.

4.7.1.3 Starch

Table 40. Starch content of fermented food mixtures on storage (g/100g)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	54.773 ^{a}	54.133 ^{b}	53.047 ^{c}	51.853 ^{d}	50.653 ^{e}	49.473 ^{f}	48.153 ^{g}
T_2	55.063 ^{a}	54.447 ^{b}	53.360 ^{c}	52.150 ^{d}	50.880 ^{e}	49.733 ^{f}	48.357 ^{g}
T_3	54.567 ^{a}	53.953 ^{b}	52.850 ^{c}	52.163 ^{d}	50.363 ^{e}	49.183 ^{f}	47.853 ^{g}
T_7	54.793 ^{a}	54.147 ^{b}	53.057 ^{c}	51.867 ^{d}	50.727 ^{e}	49.633 ^{f}	48.227 ^{g}
T_8	54.513 ^{a}	53.880 ^{b}	53.103 ^{c}	51.570 ^{d}	50.363 ^{e}	49.207 ^{f}	47.857 ^{g}
T_9	54.623 ^{a}	53.957 ^{b}	52.873 ^{c}	51.687 ^{d}	50.443 ^{e}	49.263 ^{f}	47.903 ^{g}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Starch content in FFM showed a significant decrease with the storage period. T_2 showed maximum starch content upto one month of storage (55.063 and 54.447 g/100g respectively). In the second month, T_2 showed maximum starch content (53.360g/100g), but during the third month maximum starch content was in T_3

(52.163g/100g). From fourth month onwards, T_2 showed maximum starch content upto the end of sixth month (48.357g/100g). Initially and during the first month, minimum starch content was found in T_8 (54.513 and 53.880g/100g respectively). T_3 showed the minimum starch content in the second month (52.850 g/100g), T_8 for the third (51.570g/100gm) and fourth (50.363g/100g) and T_3 for the fifth (49.183g/100g) and sixth month (47.853g/100g). Thus the initial mean value of 54.720g /100g was significantly reduced to a mean value of 48.058 g/100g after six months storage.

Table 41.Starch content of unfermented food mixtures on storage (g/100g)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	65.730 [a]	65.240 [b]	64.487 [c]	63.640 [d]	62.527 [e]	61.480 [f]	60.227 [g]
T_2	66.943 [a]	66.510 [b]	65.230 [c]	64.363 [d]	63.253 [e]	62.780 [f]	61.540 [g]
T_3	65.553 [a]	65.053 [b]	64.417 [c]	63.543 [d]	62.467 [e]	61.430 [f]	60.127 [g]
T_7	65.943 [a]	65.447 [b]	64.730 [c]	63.857 [d]	62.757 [e]	61.740 [f]	60.457 [g]
T_8	65.423 [a]	64.853 [b]	64.063 [c]	63.117 [d]	62.057 [e]	61.120 [f]	59.843 [g]
T_9	65.543 [a]	65.027 [b]	64.410 [c]	63.507 [d]	62.364 [e]	61.343 [f]	60.040 [g]

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Starch content in UFFM showed a significant reduction throughout the storage period. T_2 showed the maximum starch content throughout the storage. T_8 showed the minimum starch content initially (65.423g/100g) and throughout the storage period with 59.843g/100g after six months. Maximum starch in T_2 initially (66.943g/100g) was significantly reduced after six months storage (61.540g/100g) but showed maximum starch among UFFM treatments after storage.

Thus in all treatments UFFM , the initial mean value of starch content 65.880 g/100g showed a significant reduction throughout the storage period and reached a mean value of 60.372 g/100g towards the sixth month.

The starch content of FFM and UFFM were statistically compared by applying independent sample‘t’ test and is presented in Table 42.

Table 42 Starch content of fermented and unfermented food mixtures on storage (g/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	54.720	54.086	53.048	51.881	50.571	49.415	48.058
UFFM	65.880	65.355	64.550	63.681	62.570	61.648	60.372
Mean difference	-11.16	-11.27	-11.51	-11.79	-11.99	-12.23	-12.31
T value	-85.53	-80.10	-107.54	-113.99	-118.05	-87.06	-86.22
Significance	S	S	S	S	S	S	S

Starch content of FFM and UFFM showed a significant reduction with storage. Starch content of FFM were significantly low when compared to UFFM.

4.7.1.4. Total soluble solids (TSS)

Table 43. TSS in fermented food mixtures on storage (°Brix)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	11.66 ^{a}	11.66 ^{a}	11.68 ^{a}	11.70 ^{b}	11.72 ^{d}	11.72 ^{d}	11.75 ^{e}
T_2	11.67 ^{a}	11.68 ^{a}	11.72 ^{b}	11.74 ^{a}	11.76 ^{c}	11.77 ^{c}	11.80 ^{d}
T_3	11.65 ^{a}	11.67 ^{a}	11.68 ^{a}	11.70 ^{b}	11.73 ^{c}	11.75 ^{c}	11.79 ^{d}
T_7	11.67 ^{a}	11.68 ^{a}	11.68 ^{a}	11.71 ^{b}	11.72 ^{b}	11.74 ^{c}	11.77 ^{d}
T_8	11.67 ^{a}	11.67 ^{a}	11.68 ^{a}	11.71 ^{b}	11.72 ^{b}	11.74 ^{c}	11.76 ^{c}
T_9	11.65 ^{a}	11.66 ^{a}	11.67 ^{a}	11.69 ^{b}	11.71 ^{c}	11.71 ^{c}	11.74 ^{d}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

TSS in the FFM showed no significant difference from the initial period upto second month except in T_2. By the third month, a significant increase was observed in all the treatments. During the fourth month, there was no significant increase in T_7 and T_8 (11.72°brix). During the fifth month, a significant increase in TSS was noted in T_7 (11.74°brix) and T_8 (11.74°brix). After six months of storage, a significant increase in TSS was noted in all treatments. TSS was maximum in T_2 (11.80°brix) after the storage period. Thus the TSS of FFM increased significantly from the initial mean value of 11.66°brix to a mean value of 11.76°brix after the storage period.

Table 44. TSS in unfermented food mixtures on storage (°Brix)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	14.87 ^{g}	14.94 ^{f}	15.15 ^{e}	15.33 ^{d}	15.57 ^{c}	15.96 ^{b}	16.13 ^{a}
T_2	14.97 ^{g}	15.12 ^{f}	15.23 ^{e}	15.41 ^{d}	15.57 ^{c}	16.03 ^{b}	16.16^{a}
T_3	14.92 ^{g}	15.03 ^{f}	15.24 ^{e}	15.37 ^{d}	15.56 ^{c}	15.96 ^{b}	16.11 ^{a}
T_7	14.87 ^{g}	14.93 ^{f}	15.05 ^{e}	15.27 ^{d}	15.46 ^{c}	15.86 ^{b}	16.07 ^{a}
T_8	14.95 ^{g}	15.15 ^{f}	15.27 ^{e}	15.45 ^{d}	15.57 ^{c}	16.06 ^{b}	16.22 ^{a}
T_9	14.92 ^{g}	15.06 ^{f}	15.17 ^{e}	15.35 ^{d}	15.51 ^{c}	16.03 ^{b}	16.14 ^{a}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

TSS content in UFFM showed a significant increase throughout the storage period. Initially, T_2 showed a maximum TSS of 14.97°brix while T_1 and T_7 showed a minimum TSS (14.87°brix). TSS was maximum in T_8 in the first (15.15g/100g), second (15.27°brix), third (15.45°brix), fourth (15.57°brix), fifth (16.06°brix), and sixth (16.22°brix) month of storage. TSS was minimum in T_7 throughout the storage period. At the end of sixth month, T_7 showed a minimum TSS of 16.07°brix. The mean initial TSS was 14.92°brix which was significantly increased to a mean value of 16.14°brix after the storage period.

In both FFM and UFFM the TSS increased significantly with storage.

The TSS of FFM and UFFM were statistically compared by applying independent sample‘t’ test and is presented in Table 45.

Table 45. TSS content of fermented and unfermented food mixtures on storage (g/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	11.661	11.670	11.685	11.708	11.726	11.738	11.768
UFFM	14.916	15.038	15.185	15.363	15.540	15.598	16.138
Mean difference	-3.25	-3.36	-3.50	-3.65	-3.81	-4.24	-4.37
t value	-189.87	-19.58	-104.73	-157.43	-191.19	-136.96	-193.08
	S	S	S	S	S	S	S

TSS of FFM were significantly low when compared to UFFM throughout the storage period.

4.7.1.5. Reducing sugars and total sugars

Table 46. Reducing sugars in fermented food mixtures on storage (g/100g)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	3.07 ^{d}	3.08 ^{d}	3.10 ^{c}	3.12 ^{c}	3.14 ^{b}	3.16 ^{a}	3.17 ^{a}
T_2	2.97 ^{e}	2.98 ^{de}	2.99 ^{d}	3.01 ^{cd}	3.03 ^{bc}	3.05 ^{b}	3.07^{a}
T_3	2.76 ^{ef}	2.77 ^{ef}	2.79 ^{de}	2.81 ^{cd}	2.83 ^{bc}	2.85 ^{b}	2.87 ^{a}
T_7	3.05 ^{d}	3.05 ^{d}	3.08 ^{c}	3.10 ^{c}	3.12 ^{bc}	3.14 ^{b}	3.16 ^{a}
T_8	2.97 ^{d}	2.97 ^{d}	2.99 ^{cd}	3.03 ^{c}	3.02 ^{c}	3.04 ^{b}	3.06 ^{a}
T_9	2.93 ^{d}	2.94 ^{cd}	2.96 ^{c}	2.98 ^{b}	2.99 ^{b}	3.02 ^{a}	3.03 ^{a}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Reducing sugars in FFM showed a significant increase with the storage period. Initially maximum reducing sugar was in T_1 (3.07g/100g) and minimum in T_3 (2.76g/100g). T_1 showed maximum reducing sugar in the first (3.08 g/100g), second (3.10g/100g) third (3.12g/100g), fourth (3.14g/100g), fifth (3.16g/100g) and sixth (3.17g/100g) month of storage. There was no significant increase during the first month but significant increase was observed from the second month. During the third month the increase was not significant but later there was a significant increase till the fifth month and no significant increase in the sixth month. Minimum reducing sugar was in T_3 throughout the storage period and by the end of the sixth month, minimum reducing sugar was 2.87g/100g.

Table 47. Reducing sugars in unfermented food mixtures on storage (g/100g)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	4.76 ^{g}	4.83 ^{f}	4.96 ^{e}	5.25 ^{d}	5.35 ^{c}	5.52 ^{b}	5.73 ^{a}
T_2	4.67 ^{g}	4.75 ^{f}	4.84 ^{e}	5.17 ^{d}	5.20 ^{c}	5.45 ^{b}	5.66^{a}
T_3	4.24 ^{g}	4.36 ^{f}	4.46 ^{e}	4.85 ^{d}	5.03 ^{c}	5.31 ^{b}	5.60 ^{a}
T_7	4.96 ^{g}	5.13 ^{f}	5.27 ^{e}	5.54 ^{d}	5.68 ^{c}	5.87 ^{b}	5.97 ^{a}
T_8	4.74 ^{g}	4.86 ^{f}	4.94 ^{e}	5.26 ^{d}	5.43 ^{c}	5.73 ^{b}	5.92 ^{a}
T_9	4.72 ^{g}	4.78 ^{f}	4.86 ^{e}	5.23 ^{d}	5.36 ^{c}	5.57 ^{b}	5.72 ^{a}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Reducing sugar in UFFM showed a significant increase throughout the storage period. Initially maximum reducing sugar was in T_7 (4.96g/100g) and minimum in T_3 (4.24g/100g). T_7 showed maximum reducing sugar throughout the storage period of six months (5.97g/100g) and T_3 showed the minimum reducing sugar during storage (5.60g/100g). The initial mean reducing sugar, 4.681 g/100g was significantly increased to a mean of 5.767g/100g after six months of storage.

The reducing sugar content of FFM and UFFM were statistically compared by applying independent sample't' test and is presented in Table 48.

Table 48**.** Reducing sugars in fermented and unfermented food mixtures on storage (g/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	2.958	2.965	2.985	3.008	3.021	3.043	3.060
UFFM	4.681	4.785	4.888	5.216	5.341	5.475	5.767
Mean difference	-1.72	1.82	-1.90	-2.20	-2.32	-2.53	-2.71
t value	-16.07	-16.43	-16.46	-21.87	-21.14	-27.14	-36.31
Significance	S	S	S	S	S	S	S

In UFFM, reducing sugars were significantly high in all the storage periods when compared to FFM.

Table 49. Total sugars in fermented food mixtures on storage (g/100g)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	5.62 ^{f}	5.62 ^{f}	5.65 ^{e}	5.68 ^{d}	5.71 ^{c}	5.75 ^{b}	5.78 ^{a}
T_2	5.37 ^{f}	5.38 ^{f}	5.40 ^{e}	5.42 ^{d}	5.45 ^{c}	5.49 ^{b}	5.52 ^{a}
T_3	5.25 ^{f}	5.24 ^{f}	5.26 ^{e}	5.27 ^{d}	5.29 ^{c}	5.32 $^{\b}$	5.35 ^{a}
T_7	5.16 ^{f}	5.17^{f}	5.19 ^{e}	5.22 ^{d}	5.25 ^{c}	5.29 ^{b}	5.33 ^{a}
T_8	5.08 ^{f}	5.06^{f}	5.09 ^{e}	5.10 ^{d}	5.13 ^{c}	5.16 ^{b}	5.19 ^{a}
T_9	4.86 ^{f}	4.84 ^{f}	4.87 ^{e}	4.89 ^{d}	4.91 ^{c}	4.95 ^{b}	4.99 ^{a}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Total sugar in FFM showed a significant increase from the first month onwards. Initially maximum total sugar was in T_1 (5.62g/100g) and minimum in T_9 (4.86g/100g). T_1 showed a significant increase in total sugar throughout the storage period of six months (5.78g/100g) and T_9 showed the minimum total sugar after the sixth month of storage (4.99g/100g).There was no significant increase in the total sugar upto one month of storage in all the treatments of FFM. The mean total sugar of 5.223 g/100g initially showed a significant increase to a mean value of 5.36 g/100g by the sixth month of storage.

Table 50. Total sugars in unfermented food mixtures on storage (g/100g)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	9.96 ^{g}	10.27 ^{f}	10.14 ^{e}	10.33 ^{d}	10.44 ^{c}	10.55 ^{b}	10.72 ^{a}
T_2	9.76 ^{g}	9.85 ^{f}	9.97 ^{e}	10.17 ^{d}	10.26 ^{c}	10.37 ^{b}	10.57^{a}
T_3	9.55 ^{g}	9.65 ^{f}	9.76 ^{e}	9.94 ^{d}	10.06 ^{c}	10.17 ^{b}	10.33 ^{a}
T_7	9.57 ^{g}	9.66 ^{f}	9.81 ^{e}	10.01 ^{d}	10.16 ^{c}	10.26 ^{b}	10.42 ^{a}
T_8	9.36 ^{g}	9.49 ^{f}	9.65 ^{e}	9.94 ^{d}	10.07 ^{c}	10.22 ^{b}	10.45 ^{a}
T_9	9.04 ^{g}	9.14 ^{f}	9.35 ^{e}	9.56 ^{d}	9.87 ^{c}	10.27 ^{b}	10.28 ^{a}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Total sugar content in UFFM also showed a significant increase with the storage period. Maximum total sugar was in T_1 (9.96g/100g) and minimum in T_9 (9.04g/100g). T_1 showed maximum total sugar throughout the storage period of six months (10.72g/100g) and T_9 showed a minimum total sugar upto the fourth month of storage (9.87g/100g) but, in the fifth month it was in T_3 (10.17g/100g) and in the sixth month, it was again in T_9 (10.28g/100g). An initial mean total sugar of 9.54g/100g showed a significant increase to a mean of 10.46g/100g by the sixth month of storage.

The total sugars of FFM and UFFM were statistically compared by applying independent sample‘t’ test and is presented in Table 51.

Table 51. Total sugar in fermented and unfermented food mixtures on storage (g/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	5.223	5.220	5.240	5.26	5.29	5.33	5.36
UFFM	9.54	9.67	9.78	9.99	10.14	10.30	10.46
Mean difference	-4.31	-4.45	-4.53	-4.72	-4.85	-4.98	-5.10
t value	-25.73	-23.66	-29.18	-30.85	-35.44	-39.79	-39.53
Significance	S	S	S	S	S	S	S

In both FFM and UFFM total sugar increased significantly with storage. Total sugars in UFFM were significantly high in all the storage periods when compared to FFM.

4.7.1.6. Protein

Table 52. Protein content of fermented food mixtures on storage (g/100g)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	9.430 [a]	9.240 [b]	9.030 [c]	8.550 [d]	8.200 [e]	8.037 [f]	7.877 [g]
T_2	8.800 [a]	8.690 [b]	8.453 [c]	7.923 [d]	7.567 [e]	7.350 [f]	7.160 [g]
T_3	9.805 [a]	9.670 [b]	9.450 [c]	9.063 [d]	8.663 [e]	8.433 [f]	8.243 [g]
T_7	9.130 [a]	8.740 [b]	8.430 [c]	8.280 [d]	8.073 [e]	7.920 [f]	7.770 [g]
T_8	9.455 [a]	9.253 [b]	9.020 [c]	8.640 [d]	8.324 [e]	8.106 [f]	7.963 [g]
T_9	9.086 [a]	8.873 [b]	8.667 [c]	8.243 [d]	8.073 [e]	7.930 [f]	7.743 [g]

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Protein content in the fermented food mixtures showed a significant decrease with the storage period. Initially, T_3 (9.805 g/100g) showed a maximum protein and T_2 (8.800/100g) showed the minimum protein content. T_3 showed maximum protein in all the storage periods with protein content of 8.243g/100g at the end of the storage period. Minimum protein was shown in T_2 throughout the storage period except in the second month. T_2 showed a minimum protein of 7.160 g/100g by the end of sixth month. An initial mean protein of 9.290 g/100g showed a significant reduction to a mean value of 7.793 g/100g by the sixth month of storage.

Table 53. Protein content of unfermented food mixtures on storage (g/100g)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	7.370 [a]	7.216 [b]	7.146 [c]	6.970 [d]	6.867 [e]	6.770 [f]	6.580 [g]
T_2	6.837 [a]	6.767 [b]	6.647 [c]	6.317 [d]	6.217 [e]	6.230 [f]	6.067 [g]
T_3	7.645 [a]	7.503 [b]	7.373 [c]	7.227 [d]	7.133 [e]	7.020 [f]	6.837 [g]
T_7	7.040 [a]	6.920 [b]	6.830 [c]	6.660 [d]	6.550 [e]	6.377 [f]	6.213 [g]
T_8	7.370 [a]	7.243 [b]	7.147 [c]	7.037 [d]	6.875 [e]	6.677 [f]	6.460 [g]
T_9	7.013 [a]	6.967 [b]	6.820 [c]	6.673 [d]	6.527 [e]	6.450 [f]	6.247 [g]

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Protein content in UFFM also showed a significant decrease with the storage period. Initially, T_3 showed the maximum protein (7.645 g/100g) and T_2 (6.837/100g) the minimum protein. T_3 showed maximum protein throughout the storage with a protein content of 6.837g/100gm at the end of the storage period. Minimum protein was shown observed in T_2 throughout the storage period. T_2 showed the least protein content of 6.067 g/100g by the end of sixth month. Initially the mean protein content of 7.21g/100g reduced significantly to a mean of 6.40 g/100g after the storage period of six months.

The protein content in fermented and unfermented food mixtures were statistically compared by applying independent sample 't' test and is presented in Table 54.

Table 54. Protein in fermented and unfermented food mixtures on storage (g/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	9.290	9.026	8.895	8.451	8.150	7.962	7.793
UFFM	7.210	7.102	6.993	6.839	6.711	6.587	6.401
Mean difference	2.080	1.923	1.901	1.616	1.439	1.376	1.393
T value	20.31	16.63	19.30	15.00	13.88	13.58	13.79
Significance	S	S	S	S	S	S	S

In both FFM and UFFM, the protein decreased significantly with storage. Protein content of FFM were significantly high compared to UFFM throughout the storage period.

4.7.1.7. ß carotene

Table 55.β carotene in fermented food mixtures on storage (µg/100g)

	Storage period in months						
FFM	Initial	1	2	3	4	5	6
T_1	563.53 [a]	561.21 [b]	557.66 [c]	546.72 [d]	522.55 [e]	496.45 [f]	478.23 [g]
T_2	509.05 [a]	506.86 [b]	501.74 [c]	491.31 [d]	463.10 [e]	438.24 [f]	413.16 [g]
T_3	470.91 [a]	467.84 [b]	463.16 [c]	455.24 [d]	429.04 [e]	402.17 [f]	377.16 [g]
T_7	543.66 [a]	542.33 [b]	437.73 [c]	421.74 [d]	399.60 [e]	370.15 [f]	343.55 [g]
T_8	515.08 [a]	512.81 [b]	507.53 [c]	492.17 [d]	465.65 [e]	441.53 [f]	417.46 [g]
T_9	483.36 [a]	481.95 [b]	478.12 [c]	461.18 [d]	436.25 [e]	411.57 [f]	388.32 [g]

Values having different super script differ significantly at 5% level
DMRT row wise comparison

β carotene showed a significant reduction with storage period. Initially maximum β carotene was in T_1 (563.53µg/100g) and minimum was in T_3 (470.91µg/100g). Among treatments, T_1 showed maximum β carotene throughout the storage period and showed 478.23 ug/100g after six months of storage. T_3 showed the least β carotene content throughout the storage period and reached the least value of 377.16g/100g after the storage period.

Table 56.β carotene in unfermented food mixtures on storage (µg/100g)

	Storage period in months						
UFFM	Initial	1	2	3	4	5	6
T_1	567.49 [a]	562.35 [b]	557.73 [c]	546.85	523.14 [e]	496.53 [f]	478.30 [g]
T_2	511.35 [a]	500.15 [b]	501.32 [c]	491.76	467.72 [e]	441.43 [f]	413.54 [g]
T_3	470.62 [a]	467.57 [b]	463.16 [c]	455.22[d]	429.26 [e]	402.24 [f]	377.20 [g]
T_7	545.07 [a]	542.30 [b]	437.85 [c]	425.64	399.71 [e]	371.83 [f]	344.72 [g]
T_8	515.90 [a]	511.97 [b]	507.92	493.84	465.36 [e]	442.94 [f]	418.73 [g]
T_9	484.45 [a]	481.14 [b]	478.15[c]	46274	436.36 [e]	411.74 [f]	389.24 [g]

Values having different super script differ significantly at 5% level
DMRT row wise comparison

β carotene in UFFM also showed a significant decrease with storage period. Initially maximum β carotene was in T_1 (567.49µg/100g) and the minimum was in T_3 (470.62µg/100g). T_1 showed maximum β carotene throughout the storage period among the 6 treatments, with 478.30µg/100g after six months of storage. T_3 showed the least β carotene content among all the treatments throughout the storage period, with the least value of 377.20 µg/100g after six months.

The β carotene content of FFM and UFFM were statistically compared by applying independent sample't' test and is presented in Table 57.

Table 57. β carotene content of fermented and unfermented food mixtures on storage (µg/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	514.25	512.14	490.98	476.55	452.07	426.68	402.97
UFFM	515.80	510.90	490.52	479.51	453.59	427.95	403.63
Mean difference	-1.54	1.25	0.461	-2.95	-0.89	-0.126	-0.655
T value	-0.138	-0.112	-0.036	-0.0217	-0.067	-0.094	-0.046
Significance	0.891	0.911	0.972	0.829	0.947	0.925	0.964
	NS	NS	NS	NS	NS	NS	NS

There was a significant reduction in the β carotene content of FFM and UFFM during storage, but the variation observed in the β carotene content of FFM and UFFM was not significant.

4.7.1.8. Calcium

Table 58. Calcium content in fermented food mixtures on storage (mg/100g)

	Storage period in months						
FFM	Initial	1	2	3	4	5	6
T_1	67.77 ^{a}	67.43 ^{b}	66.53 ^{c}	64.25 ^{d}	62.12 ^{e}	60.42 ^{f}	59.16 ^{g}
T_2	67.31 ^{a}	67.18 ^{b}	66.23 ^{c}	63.94 ^{d}	61.84 ^{e}	60.11 ^{f}	58.81 ^{g}
T_3	69.70 ^{a}	69.37 ^{b}	68.41 ^{c}	66.12 ^{c}	63.87 ^{e}	62.20 ^{f}	60.96 ^{g}
T_7	68.25 ^{a}	67.92 ^{b}	67.03 ^{c}	64.82 ^{d}	62.64 ^{e}	60.93 ^{f}	59.66 ^{g}
T_8	66.91 ^{a}	66.65 ^{b}	65.68 ^{c}	63.46 ^{d}	61.34 ^{e}	59.68 ^{f}	58.44 ^{g}
T_9	69.06 ^{a}	68.75 ^{b}	67.81 ^{c}	65.56 ^{d}	63.46 ^{e}	61.76 ^{f}	60.46 ^{g}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Calcium content in the FFM showed a significant reduction with storage period. T_3 showed maximum calcium content initially (69.70mg/100g) and minimum calcium was seen in T_8 (66.91mg/100g). Maximum calcium content was observed in T_3 throughout the storage period till the end of sixth month (60.96 mg/100g).The least calcium after sixth month was observed in T_8 (58.44 mg/100g). The mean initial value of 68.167mg/100g calcium was significantly reduced to 59.58 mg/100g after six months of storage.

Table 59.Calcium content of unfermented food mixtures on storage (mg/100g)

	Storage period in months						
UFFM	Initial	1	2	3	4	5	6
T_1	67.69 ^{a}	67.38 ^{b}	66.43 ^{c}	64.08 ^{d}	61.82 ^{e}	60.63 ^{f}	59.02 ^{g}
T_2	67.28 ^{a}	67.16 ^{b}	66.11 ^{c}	63.41 ^{d}	61.35 ^{e}	60.01 ^{f}	59.11 ^{g}
T_3	69.23 ^{a}	69.05 ^{b}	68.30 ^{c}	65.59 ^{c}	63.63 ^{e}	62.15 ^{f}	60.53 ^{g}
T_7	67.40 ^{a}	67.21 ^{b}	66.90 ^{c}	64.58 ^{d}	61.54 ^{e}	60.53 ^{f}	59.29 ^{g}
T_8	66.55 ^{a}	66.38 ^{b}	65.45 ^{c}	63.07 ^{d}	60.91 ^{e}	59.68 ^{f}	58.16 ^{g}
T_9	68.84 ^{a}	67.62 ^{b}	66.52 ^{c}	64.87 ^{d}	61.71 ^{e}	60.61 ^{f}	59.43 ^{g}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Calcium content in UFFM also showed a significant reduction with storage period. Among all the treatments, T_3 showed a maximum calcium initially (69.23mg/100g) and minimum was observed in T_8 (66.55mg/100g). Maximum calcium content was showed in T_3 throughout the storage period till the end of the sixth month (60.53 mg/100g) and minimum was in T_8 (58.16mg/100g). The initial mean calcium content of 67.83mg/100g showed a significant reduction to 59.29 mg/100g towards the end of sixth month.

The calcium content of FFM and UFFM were statistically compared by applying independent sample‘t’ test and is presented in Table 60.

Table 60. Calcium content of fermented and unfermented food mixtures on storage (mg/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	68.17	67.88	66.94	64.67	62.54	60.84	59.58
UFFM	67.82	67.29	66.62	64.25	61.82	60.59	59.29
Mean difference	0.343	0.587	0.317	0.435	0.718	0.250	0.287
t value	1.680	1.860	1.016	1.428	2.396	0.877	1.077
Significance	0.878	0.577	0.423	0.675	0.350	0.138	.049
	NS	NS	NS	NS	NS	NS	NS

There was no significant variation in the calcium content of fermented and unfermented food mixtures during storage.

4.7.1.9 .Potassium

Table 61.Potassium content in fermented food mixtures on storage (mg/100g)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	305.33 [a]	305.06 [b]	304.65 [c]	302.25 [d]	300.15 [e]	298.64 [f]	287.54 [g]
T_2	304.67 [a]	304.32 [b]	304.02 [c]	301.13 [d]	300.00 [e]	297.95 [f]	286.33 [g]
T_3	396.67 [a]	396.26 [b]	396.04 [c]	393.05 [d]	392.15 [e]	389.95 [f]	377.82 [g]
T_7	307.33 [a]	307.00 [b]	306.68 [c]	304.33 [d]	302.16 [e]	299.86 [f]	287.66 [g]
T_8	313.67 [a]	313.25 [b]	312.97 [c]	311.03 [d]	308.96 [e]	306.54 [f]	293.06 [g]
T_9	306.00 [a]	305.84 [b]	305.54 [c]	303.35 [d]	301.24 [e]	298.85 [f]	282.56 [g]

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Potassium content in FFM showed a significant reduction with storage period. Initially, T_3 (396.67mg/100g) showed the maximum potassium content and minimum was observed in T_2 (304.67 mg/100g). Maximum calcium content was in T_3 throughout the storage period till the end of sixth month (377.82mg/100g). The least potassium was observed in T_9 (282.56mg/100g) after six months. The mean value of (325.27 mg/100g) potassium during the initial period showed a significant reduction to 302.49 mg/100g after six months of storage.

Table 62. Potassium content in unfermented food mixtures on storage (mg/100g)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	304.33 [a]	304.02 [b]	303.57 [c]	301.12 [d]	299.96 [e]	298.13 [f]	286.75 [g]
T_2	304.33 [a]	304.03 [b]	303.55 [c]	301.05 [d]	299.74 [e]	298.04 [f]	286.23 [g]
T_3	396.33 [a]	396.02 [b]	396.05 [c]	393.55 [d]	391.18 [e]	389.27 [f]	378.22 [g]
T_7	307.00 [a]	306.22 [b]	306.54 [c]	304.13 [d]	302.10 [e]	299.44 [f]	287.14 [g]
T_8	313.00 [a]	312.77 [b]	312.41 [c]	310.25 [d]	307.97 [e]	305.94 [f]	290.15 [g]
T_9	302.67 [a]	302.33 [b]	302.06 [c]	300.15 [d]	298.83 [e]	298.84 [f]	280.76 [g]

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Potassium content in UFFM also showed a significant reduction with storage period. Among all the unfermented treatments, T_3 showed a maximum initial potassium content (396.33mg/100g) and minimum was observed in T_9 (302.67 mg/100g). Maximum calcium was in T_3 throughout the storage period till the end of sixth month (378.22mg/100g). The least potassium content was in T_9 (280.76mg/100g) after six months. The mean initial value of potassium which was 321.27mg/100g was significantly reduced to a mean value of 301.54 mg/100g after six months of storage.

The potassium content of FFM and UFFM were statistically compared by applying independent sample‘t’ test and is presented in Table 63.

Table 63. Potassium content in fermented and unfermented food mixtures on storage (mg/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	325.27	325.45	316.45	319.52	317.44	315.29	302.49
UFFM	321.27	333.48	331.22	324.50	322.29	370.90	301.54
Mean difference	4.001	-0.035	-14.521	-4.97	-5.35	-5.60	-6.17
t value	0.343	-0.487	-1.280	-0.454	-0.489	-0.514	-0.595
Significance	0.733	0.629	0.209	0.653	0.628	0.610	0.590
	NS	NS	NS	NS	NS	NS	NS

Potassium content in FFM and UFFM showed no significant difference during storage.

4.7.1.10. Iron

Table 64. Iron content in fermented food mixtures on storage (mg/100g)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	6.99 [a]	6.73 [b]	6.65 [c]	6.36 [d]	6.36 [d]	6.12 [f]	5.96 [g]
T_2	6.33 [a]	6.15 [b]	6.03 [c]	5.82 [d]	5.77 [e]	5.42 [f]	5.26 [g]
T_3	6.79 [a]	6.55 [b]	6.37 [c]	6.17 [d]	6.06 [e]	5.87 [f]	5.66 [g]
T_7	6.13 [a]	6.00 [b]	5.86 [c]	5.65 [d]	5.37 [e]	5.07 [f]	4.94 [g]
T_8	6.26 [a]	6.04 [b]	5.88 [c]	5.60 [d]	5.32 [e]	5.15 [f]	5.04 [g]
T_9	6.24 [a]	6.07 [b]	5.86 [c]	5.66 [d]	5.33 [e]	5.18 [f]	5.08 [g]

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Iron content in FFM showed a significant reduction with storage period. T_1 had maximum iron content during the initial period (6.99mg/100g) and minimum was in T_7 (6.13 mg/100g). Eventhough there was a significant reduction in iron content, maximum iron retention was found in T_1 throughout the study and showed a value of 5.96 mg/100g after six months. Similarly T_7 showed the least iron content throughout the storage, and finally showed an iron value of 4.94 mg/100g after six months .The initial mean value of 6.45 mg/100g was significantly reduced to 5.32 mg/100g after six months.

Table 65. Iron content in unfermented food mixtures on storage (mg/100g)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	6.90 [a]	6.73 [a]	6.65 [b]	6.39 [c]	6.34 [d]	6.05 [e]	5.93 [f]
T_2	6.32 [a]	6.13 [a]	5.94 [b]	5.86 [c]	5.61 [d]	5.36 [e]	5.14 [f]
T_3	6.77 [a]	6.50 [a]	6.35 [b]	6.12 [c]	5.93 [d]	5.82 [e]	5.66 [f]
T_7	6.17 [a]	5.96 [a]	5.82 [b]	5.68 [c]	5.40 [d]	5.03 [e]	4.83 [f]
T_8	6.26 [a]	6.04 [a]	5.87 [b]	5.57 [c]	5.27 [d]	5.12 [e]	4.96 [f]
T_9	6.21 [a]	6.01 [a]	5.83 [b]	5.60 [c]	5.31 [d]	5.07 [e]	4.88 [f]

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Iron content in UFFM also showed a significant reduction with storage period. Among all treatments of UFFM initially, T_1 (6.90mg/100g) showed the maximum iron

content and minimum was seen in T_7 (6.17 mg/100g). Maximum iron content was in T_1 throughout the storage period with iron content of 5.93mg/100g during the sixth month. The least value of iron was in T_7 initially, in the first month (5.96mg/100g) and second month (5.82mg/100g), but in the fourth month, least iron was in T_8 (5.27mg/100g). T_7 showed the least iron during the fifth month (5.03 mg/100g) and sixth month (4.83 mg/100g). The initial mean value of 6.44 mg/100g was significantly reduced to 5.23 mg/100g after six months of storage in UFFM.

The iron content of FFM and UFFM were statistically compared by applying independent sample‘t’ test and is presented in Table 66.

Table 66. Iron content in fermented and unfermented food mixtures on storage (mg/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	6.45	6.25	6.10	5.87	5.63	5.47	5.32
UFFM	6.44	6.22	6.08	5.57	5.64	5.40	5.23
Mean difference	0.013	0.031	0.027	0.008	0.051	0.0622	0.009
t value	0.126	0.314	0.258	0.683	0.179	0.460	0.691
Significance	0.901	0.756	0.798	0.934	0.707	0.648	0.498
	NS	NS	NS	NS	NS	NS	NS

There was no significant variation in the iron content of FFM and UFFM during storage.

4.7.1.11. Thiamine

Table 67. Thiamine content of fermented food mixtures on storage (mg/100g)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	0.085^{a}	0.067^{b}	0.055^{c}	0.047^{d}	0.040^{e}	0.026^{f}	0.018^{g}
T_2	0.073^{a}	0.061^{b}	0.050^{c}	0.041^{d}	0.035^{e}	0.021^{f}	0.012^{g}
T_3	0.089^{a}	0.069b	0.058^{c}	0.051^{d}	0.044^{e}	0.029^{f}	0.023^{g}
T_7	0.083^{a}	0.063^{b}	0.054^{c}	0.050^{d}	0.043^{e}	0.026^{f}	0.017^{g}
T_8	0.085^{a}	0.067^{b}	0.056^{c}	0.051^{d}	0.044^{e}	0.028^{f}	0.019^{g}
T_9	0.075^{a}	0.063^{b}	0.053^{c}	0.043^{d}	0.038^{e}	0.023^{f}	0.012^{g}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant reduction in the thiamine content of FFM during storage. Thiamine content varied from 0.073 to 0.089 mg/100g in the initial period the maximum in T_3. After six months of storage, it showed a variation from 0.012 to 0.019 mg/100g and the maximum was in T_8.

Table 68. Thiamine content of unfermented food mixtures on storage (mg/100g)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	0.066^a	0.058^b	0.047^c	0.033^d	0.028^e	0.015^f	0.011^g
T_2	0.053^a	0.050^b	0.041^c	0.027^d	0.019^e	0.007^f	0.013^g
T_3	0.056^a	0.048b	0.037^c	0.025^d	0.016^e	0.005^f	0.002^g
T_7	0.064^a	0.056^b	0.045^c	0.031^d	0.022^e	0.003^f	0.001^g
T_8	0.068^a	0.057^b	0.047^c	0.031^d	0.023^e	0.003^f	0.001^g
T_9	0.055^a	0.044^b	0.033^c	0.021^d	0.018^e	0.002^f	0.001^g

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Thiamine content in UFFM showed a significant reduction with storage. Initially, maximum thiamine was in T_8 (0.068 mg/100g) and minimum in T_2 (0.053 mg/100g). After six months maximum thiamine was in T_2 (0.013 mg/100g) and least in T_7, T_8 and T_9 (0.001 mg/100g).

The thiamine content of FFM and UFFM were statistically compared by applying independent sample‘t’ test and is presented in Table 69.

Table 69. Thiamine content of fermented and unfermented food mixtures on storage (mg/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	0.0817	0.065	0.054	0.047	0.041	0.025	0.016
UFFM	0.0603	0.052	0.041	0.028	0.021	0.005	0.003
Mean difference	0.021	0.012	0.013	0.019	0.019	0.020	0.014
T value	5.82	4.86	5.02	7.51	8.52	8.45	5.78
Significance	S	S	S	S	S	S	S

Thiamine content in FFM were found to be significantly high when compared to UFFM.

4.7.1.12. Riboflavin

Table 70. Riboflavin content of fermented food mixtures on storage (mg/100g)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	0.65^a	0.44^b	0.38^c	0.36^d	0.34^e	0.28^f	0.18^g
T_2	0.61^a	0.40^b	0.32^c	0.31^d	0.25^e	0.21^f	0.14^g
T_3	0.61^a	0.41^b	0.34^c	0.33^d	0.28^e	0.24^f	0.15^g
T_7	0.62^a	0.42^b	0.35^c	0.33^d	0.30^e	0.25^f	0.17^g
T_8	0.64^a	0.44^b	0.36^c	0.35^d	0.32^e	0.26^f	0.16^g
T_9	0.61^a	0.40^b	0.32^c	0.31^d	0.25^e	0.22^f	0.15^g

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Riboflavin content of FFM also reduced significantly with storage period. Initially, riboflavin which varied from 0.61 to 0.65 mg/100g was significantly reduced to 0.14 to 0.18 by the sixth month. Initially, maximum riboflavin was in T_1 (0.65 mg/100g) and the least in T_9, T_3 and T_2 (0.61 mg/100g). After six months, maximum was in T_1 (0.18 mg/100g) and the least was in T_2 (0.14 mg/100g).

Table 71. Riboflavin content of unfermented food mixtures on storage (mg/100g)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	0.34^a	0.28^b	0.22^c	0.15^d	0.11^e	0.07^f	0.02^g
T_2	0.31^a	0.25^b	0.21^c	0.12^d	0.09^e	0.05^f	0.01^g
T_3	0.31^a	0.24^b	0.21^c	0.11^d	0.07^e	0.04^f	0.007^g
T_7	0.34^a	0.26^b	0.23^c	0.14^d	0.09^e	0.05^f	0.007^g
T_8	0.36^a	0.30^b	0.24^c	0.14^d	0.08^e	0.04^f	0.005^g
T_9	0.33^a	0.28^b	0.21^c	0.12^d	0.08^e	0.02^f	0.004^g

Values having different super script differ significantly at 5% level
DMRT row wise comparison

Riboflavin in UFFM also showed a significant reduction with storage. Initially, maximum riboflavin was in T_8 (0.36 mg /100g) and minimum was in T_2 (0.31

mg/100g). After six months, maximum was in T_1 (0.02 mg/100g) and the least in T_9 (0.004 mg/100g)

The riboflavin content of FFM and UFFM were statistically compared by applying‘t’ test and is presented in Table 72.

Table 72. Riboflavin content of fermented and unfermented food mixtures on storage (mg/100g)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	0.623	0.418	0.345	0.332	0.290	0.243	0.160
UFFM	0.331	0.267	0.220	0.130	0.086	0.045	0.008
Mean difference	0.291	0.150	0.125	0.201	0.203	0.198	0.115
t value	27.33	12.72	11.49	19.27	12.66	15.87	26.57
Significance	S	S	S	S	S	S	S

Riboflavin content of FFM were found to be significantly high during all storage periods.

4.7.2. *In vitro* starch digestibility (IVSD)

Table 73. IVSD of fermented food mixtures on storage (per cent)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	80.40 ^{e}	80.43 ^{e}	80.70 ^{d}	80.77 ^{d}	81.47 ^{c}	81.75 ^{b}	82.40 ^{a}
T_2	78.37 ^{d}	78.53 ^{d}	78.56 ^{d}	80.37 ^{c}	80.46 ^{bc}	80.63 ^{b}	81.60 ^{a}
T_3	80.63 ^{e}	80.70 ^{e}	81.20 ^{d}	81.40 ^{d}	82.33 ^{c}	82.63 ^{b}	83.23 ^{a}
T_7	81.50 ^{e}	81.56 ^{e}	82.06 ^{d}	82.27 ^{d}	83.20 ^{c}	83.60 ^{b}	84.48 ^{a}
T_8	82.47 ^{d}	82.50 ^{d}	83.37 ^{c}	83.50 ^{c}	83.63 ^{c}	84.03 ^{b}	84.70 ^{a}
T_9	82.66 ^{e}	82.87 ^{e}	83.30 ^{d}	83.73 ^{c}	84.52 ^{b}	84.61 ^{b}	85.46 ^{a}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

IVSD in FFM showed a significant increase with the storage period. Initially maximum IVSD was in T_9 (82.6 per cent) and minimum in T_2 (78.37 per cent). T_9 and T_8 showed maximum IVSD during the first and second month of storage (82.87 and 83.37 per cent respectively). From the third month onwards, maximum IVSD was

observed in T_9 with 85.46 per cent after of six months. The least IVSD was observed in T_2 (81.69 per cent) after six months. There was no significant variation in IVSD of all treatments during the first month, but for T_2 significant increase was not observed even in the second month. The mean IVSD of FFM observed initially (80.98 per cent) was found to increase significantly (83.64 per cent) after six months of storage.

Table 74. IVSD of unfermented food mixtures on storage (per cent)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	55.16 ^{f}	55.17 ^{f}	55.25 ^{e}	55.43 ^{d}	55.93 ^{c}	56.17 ^{b}	56.95^{a}
T_2	54.45 ^{g}	54.50 ^{f}	54.63 ^{e}	54.96 ^{d}	53.07 ^{c}	55.36 ^{b}	56.15 ^{a}
T_3	55.02 ^{f}	55.11 ^{e}	55.16 ^{d}	55.26 ^{c}	55.46 ^{b}	55.93 ^{a}	55.97 ^{a}
T_7	54.45 ^{f}	54.46 ^{f}	54.74 ^{e}	55.24 ^{d}	55.74 ^{c}	56.06 ^{b}	56.95 ^{a}
T_8	56.01 ^{g}	56.15 ^{f}	56.33 ^{e}	56.87 ^{d}	57.34 ^{c}	57.55 ^{b}	58.23 ^{a}
T_9	56.34 ^{f}	56.63 ^{e}	56.75 ^{e}	57.03 ^{d}	57.55 ^{c}	57.76 ^{b}	58.35 ^{a}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

IVSD of UFFM showed a significant increase with the storage period. Initial IVSD varied from 54.45 to 56.34 per cent, with maximum in T_9 and minimum in T_2. T_9 showed maximum IVSD till the end of sixth month (58.35 per cent) .IVSD was least in T_7 (54.50 per cent) in the first month .After that, least IVSD was observed in T_2 throughout till the sixth month (56.15 per cent) .The initial mean value of 55.25 per cent for IVSD showed a significant increase to a mean value of 57.09 per cent after six months of storage.

The IVSD of FFM and UFFM were statistically compared by applying‘t’ test and is presented in Table 75.

Table 75. IVSD of fermented and unfermented food mixtures on storage (per cent)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	80.98	81.12	81.41	82.21	82.59	82.90	83.64
UFFM	55.25	55.32	55.48	55.74	56.25	56.18	57.09
Mean difference	25.73	25.80	25.92	26.46	26.33	26.72	26.55
t value	66.02	61.05	61.44	75.66	66.32	64.49	66.37
Significance	S	S	S	S	S	S	S

IVSD of FFM were significantly high in all storage periods compared to UFFM.

4.7.3. *In vitro* protein digestibility (IVPD)

Table 76. IVPD of fermented food mixtures on storage (per cent)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	85.14^{f}	85.16^{f}	85.26^{e}	85.64^{d}	86.15^{c}	86.23^{b}	86.75^{a}
T_2	85.83^{f}	85.85^{f}	85.96^{e}	85.23^{d}	86.96^{c}	87.05^{b}	87.55^{a}
T_3	85.56^{f}	85.58^{f}	85.85^{e}	86.21^{d}	86.94^{c}	87.13^{b}	87.59^{a}
T_7	85.74^{f}	85.76^{f}	85.93^{e}	86.14^{d}	86.85^{c}	86.97^{b}	87.16^{a}
T_8	85.15^{f}	85.18^{f}	85.44^{e}	85.85^{d}	86.46^{c}	86.64^{b}	86.84^{a}
T_9	85.55^{f}	85.55^{f}	85.76^{e}	86.14^{d}	86.90^{c}	87.05^{b}	87.31^{a}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

IVPD of the FFM showed a significant increase with the storage period. There was no significant difference in the digestibility upto one month of storage. IVPD was maximum in T_2 (85.85 per cent) and minimum in T_1 (85.16 per cent) during the second month. There was a significant increase during the third month in all the treatments, with maximum in T_3 (86.21 per cent) and minimum in T_1 (85.23 per cent). T_2 showed maximum IVPD in the fourth month (86.96 per cent) and T_3 showed maximum in the fifth and sixth month (87.13 and 87.59 per cent respectively). Least IVPD was in T_1 in the fourth (86.14 per cent) month till the end of sixth month (86.75 per cent).

Table 77. IVPD of unfermented food mixtures on storage (per cent)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	57.15^{f}	57.24^{f}	57.54^{e}	57.85^{d}	58.18^{c}	58.43^{b}	58.93^{a}
T_2	57.78^{f}	57.79^{f}	57.94^{e}	58.15^{d}	58.46^{c}	58.94^{b}	59.56^{a}
T_3	57.23^{f}	57.35^{f}	57.64^{d}	57.95^{c}	58.46^{b}	58.56^{a}	59.15^{a}
T_7	57.56^{f}	57.63^{f}	57.83^{e}	57.85^{d}	58.75^{c}	58.86^{b}	59.44^{a}
T_8	57.15^{f}	57.15^{f}	57.62^{e}	57.87^{d}	58.16^{c}	58.33^{b}	58.94^{a}
T_9	57.44^{f}	57.45^{f}	57.66^{e}	57.94^{d}	58.31^{c}	58.46^{b}	58.85^{a}

Values having different super script differ significantly at 5% level
DMRT row wise comparison

IVPD in UFFM showed a significant increase with the storage period. In all the treatments, there was no significant difference in IVPD upto one month of storage. IVPD was maximum in T_2 in the second and third month of storage (57.94 and 58.15 per cent respectively). IVPD was minimum in T_8 in the initial and first month of storage (57.15 per cent). During the third month, maximum IVPD was in T_2 (58.15 per cent). After six months of storage, IVPD was maximum in T_2 (59.56 per cent) and least in T_9 (58.85 per cent).

Initial mean IVPD of 57.39 percent was significantly increased to a mean of 59.15 percent after six months of storage.

In both FFM and UFFM *in vitro* protein digestibility increased significantly with storage.

The IVPD of FFM and UFFM were statistically compared by applying 't' test and is presented in Table 78.

Table 78. IVPD of fermented and unfermented food mixtures on storage (per cent)

	Storage period in months						
	Initial	1	2	3	4	5	6
FFM	85.49	85.53	85.69	86.03	86.71	86.84	8719
UFFM	57.38	57.43	57.70	57.94	58.38	58.50	59.14
Mean difference	28.10	28.09	27.99	28.08	28.32	28.26	28.05
t value	32.730	34.113	38.663	48.465	32.252	29.329	27.583
Significance	S	S	S	S	S	S	S

IVPD in FFM were found to be significantly high during all storage periods compared to UFFM.

4.7.4. Total microbial population and viable count of *L. acidophilus* in food mixtures on storage

4.7.4.1. Total microbial population in the fermented and unfermented food mixtures on storage.

FFM and UFFM were enumerated for total bacteria, fungi and yeast during each month of storage and the results are presented in Tables 79 to 83.

4.7.4.1.1. Total bacterial count in fermented and unfermented food mixtures on storage

Table 79.Total bacterial count in fermented food mixtures on storage (x 10^7cfu/g)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	149[a] (9.17)	144 [b] (9.16)	135 [c] (9.13)	126 [d] (9.10)	115 [e] (9.06)	108 [f] (9.03)	98 [g] (8.99)
T_2	140[a] (9.15)	133 [b] (9.12)	126 [c] (9.10)	114 [d] (9.06)	109 [e] (9.04)	96 [f] (8.98)	92 [g] (8.96)
T_3	283[a] (9.45)	273 [b] (9.44)	257 [c] (9.41)	223 [d] (9.35)	207 [e] (9.32)	189 [f] (9.28)	135[g] (9.13)
T_7	139[a] (9.14)	126 [b] (9.10)	114 [c] (9.06)	98 [d] (8.99)	91 [e] (8.96)	81 [f] (8.91)	74[g] (8.87)
T_8	211[a] (9.32)	200 [b] (9.30)	189 [c] (9.28)	173 [d] (9.24)	159 [e] (9.20)	144 [f] (9.16)	123[g] (9.09)
T_9	206[a] (9.31)	192 [b] (9.28)	177 [c] (9.25)	166 [d] (9.22)	147 [e] (9.17)	128 [f] (9.11)	110 [g] (9.04)

(Figures in parenthesis indicate log cfu/g)
Values are mean of three independent enumerations, DMRT row wise comparison
Values with different superscript differ significantly at 5 % level

Initially, total bacterial population varied from 139 to 283 (x 10^7cfu/g). Maximum bacterial count was observed in T_3 and the minimum in T_7. There was a significant reduction in the total bacterial count in FFM during storage. After six months, total bacterial count was significantly reduced which varied from 74 to 135 (x 10^7cfu/g). Maximum total bacterial population was in T_3 and minimum in T_2.

Table 80.Total bacterial count in unfermented food mixtures on storage (x 10^7cfu/g)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	3.1 [a]	3.6 [b]	4.5 [c]	5.3 [d]	6.7 [e]	7.5 [f]	8.6 [g]
T_2	3.2 [a]	3.7 [b]	4.5 [c]	5.2 [d]	6.8 [e]	7.7 [f]	8.8 [g]
T_3	3.2 [a]	3.6 [b]	4.4 [c]	5.2 [d]	6.7 [e]	7.4 [f]	8.4 [g]
T_7	3.3 [a]	3.8 [b]	5.0 [c]	5.9 [d]	7.2 [e]	8.0 [f]	9.2 [g]
T_8	3.4 [a]	3.8 [b]	4.8 [c]	5.5 [d]	7.0 [e]	7.9 [f]	9.0 [g]
T_9	3.0 [a]	3.6 [b]	4.5 [c]	5.4 [d]	6.8 [e]	7.8 [f]	9.0 [g]

Values are mean of three independent enumeration, DMRT row wise comparison
Values with different superscript differ significantly at 5 % level

Initially, total bacterial population varied from 3.0 to 3.4 x 10^7cfu/g .Maximum bacterial count was observed in T_8 and the minimum in T_9. There was an increase in the total bacterial count in UFFM during storage. After six months, total bacterial count increased which varied from 8.4 to 9.2 x 10^7cfu/g. Maximum total bacterial count was in T_7 and minimum in T_3.

4.7.4.1.2. Fungal count in fermented and unfermented food mixtures on storage

Table 81. Fungal count in fermented food mixtures on storage (x 10^3cfu/g)

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	Nil	Nil	1.0	1.3	1.8	2.0	2.1
T_2	1.0	1.0	1.3	1.5	2.0	2.1	2.3
T_3	Nil	1.0	1.0	1.3	1.5	2.0	2.1
T_7	Nil	1.0	1.0	1.3	1.8	2.0	2.1
T_8	1.0	1.5	1.6	1.8	2.0	2.1	2.3
T_9	Nil	Nil	1.0	1.5	2.0	2.1	2.3

Values are mean of three independent enumerations

Initially, fungal population was nil in T_1, T_3, T_7 and T_9 and a fungal count of 1 x10^3 cfu/g was observed in T_2 and T_8. There was an increase in the fungal count in FFM during storage. After six months, fungal count increased, which varied from 2.1 to 2.3 x 10^3cfu/g.

Table 82. Fungal count in unfermented food mixtures on storage (x 10^3cfu/g)

UFFM	Storage period in months						
	Initial	1	2	3	4	5	6
T_1	1.0	3.3	4.3	5.5	6.9	8.3	9.1
T_2	1.5	3.2	4.5	5.7	7.3	8.9	9.7
T_3	Nil	3.3	4.9	5.8	7.8	8.1	8.7
T_7	2.0	4.1	4.8	6.1	8.0	8.5	9.9
T_8	1.0	3.6	4.8	6.3	7.5	7.9	8.7
T_9	Nil	3.7	4.6	5.5	7.1	8.0	9.1

Values are mean of three independent enumerations

Initially, there was no fungal growth in T_3 and T_9 but the fungal population varied from 1.0 to 2.0 x 10^3cfu/g in the other treatments. There was an increase in the fungal count in UFFM during storage. After six months, fungal count increased, which varied from 8.7 to 9.1x 10^3 cfu/g . Maximum fungal growth was in T_2 and minimum in T3 and T8.

4.7.4.1.3. Yeast count in fermented and unfermented food mixtures on storage

There were no traces of yeast in FFM and UFFM throughout the storage period.

4.7.4.1.4. Insect infestation of the food mixtures on storage

By visual observation of the food mixtures and also by examining the mixtures under the microscope, presence of any storage insects were assessed. The powder was sieved first with 60BL sieve and followed by 100BL sieve and observed under microscope.

Insect infestation of the food mixtures was evaluated and no insect infestation was observed in the sample upto a period of six months.

4.7.4.2. Viable count of *L..acidophilus* in fermented food mixtures on storage

Table 83.Viable count of *L. acidophilus* in fermented food mixtures on storage (x 10^7cfu / g)

	Storage period in months						
FFM	Initial	1	2	3	4	5	6
T_1	147[a] (9.16)	142 [b] (9.15)	132 [c] (9.12)	123 [d] (9.08)	112 [e] (9.04)	105 [f] (9.02)	95 [g] (8.97)
T_2	139[a] (9.14)	132 [b] (9.12)	124 [c] (9.09)	112 [d] (9.05)	106 [e] (9.03)	93 [f] (8.96)	89 [g] (8.94)
T_3	282[a] (9.45)	271 [b] (9.43)	255 [c] (9.40)	221 [d] (9.34)	205 [e] (9.31)	187 [f] (9.27)	133[g] (9.12)
T_7	137[a] (9.13)	124 [b] (9.09)	111 [c] (9.04)	95 [d] (8.97)	88 [e] (8.94)	77 [f] (8.86)	70 [g] (8.84)
T_8	210[a] (9.32)	198 [b] (9.29)	187 [c] (9.27)	171 [d] (9.23)	156 [e] (9.19)	141 [f] (9.14)	120[g] (9.08)
T_9	205[a] (9.31)	191 [b] (9.28)	175 [c] (9.21)	164 [d] (9.21)	144 [e] (9.16)	125 [f] (9.09)	106 [g] (9.03)

(Figures in parenthesis are log cfu/g)
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant reduction in the viable count of *L. acidophilus* throughout the storage period. Initially, viable counts of *L. acidophilus* varied from 137 (T_7) to 282 (T_3) x 10^7cfu /g. After six months of storage, viable count was significantly reduced which varied from 70 (T_7) to 133 (T_3) x 10^7cfu /g (Fig 26).

4.7.5. Organoleptic qualities of the selected six food mixtures on storage

The results of the organoleptic qualities of the selected six food mixtures are presented in Table 84 to 89.

4.7.5.1. Organoleptic qualities of fermented and unfermented food mixtures on storage- T_1

As revealed in Table 84, initially in T_1, the mean score for appearance in FFM and UFFM was 8.26 which showed no difference in the first month also. A gradual reduction in the mean score was observed from third month onwards and after six months, the score was reduced to 8.00 in both the samples. With respect to colour, FFM had a higher score of 8.46 than UFFM (8.36) during the initial period, which on storage was decreased to 8.13 and 8.10 after six months. The mean score for flavour of FFM and UFFM showed a marked difference with 7.13 and 5.63 initially, which was reduced to 6.60 and 5.13 after six months. Initially UFFM showed a better mean score for texture (8.30) than FFM (8.23), but by the third month onwards the mean score for texture was reduced to 8.20 in both FFM and UFFM. After six months, the mean score for texture of FFM (7.86) was higher than UFFM (7.85).With regard to taste, FFM had a higher score of 7.30 initially which decreased on storage to 6.53 after six months. The mean score for taste of UFFM was 5.46 initially which decreased on storage, and after six months the mean score was 4.83. The overall acceptability was higher in FFM (7.42) after six months storage.

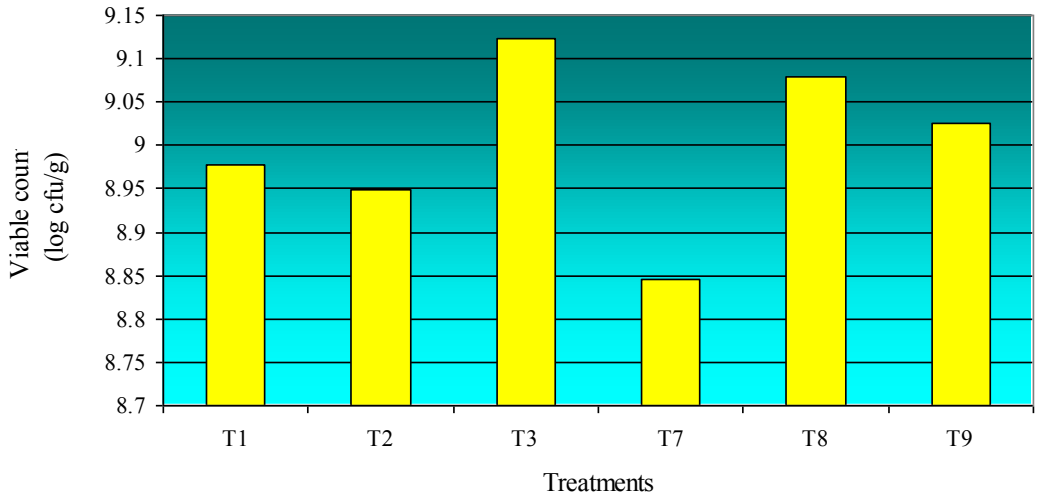


Fig. 26. Viable count of *L.acidophilus* in FFM after six months of storage

Table 84. Mean score for organoleptic qualities of fermented and unfermented food mixture on storage.-T1

Quality attributes	Storage period in months													
	Initial		1		2		3		4		5		6	
	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM
Appearance	8.26	8.26	8.26	8.26	8.26	8.25	8.20	8.20	8.13	8.10	8.01	8.02	8.00	8.00
Colour	8.46	8.36	8.45	8.36	8.45	8.36	8.40	8.36	8.33	8.30	8.30	8.26	8.13	8.10
Flavour	7.13	5.63	7.13	5.62	7.00	5.55	6.96	5.50	6.83	5.38	6.70	5.22	6.60	5.13
Texture	8.23	8.30	8.23	8.25	8.22	8.25	8.20	8.20	8.10	8.10	8.00	8.00	7.86	7.85
Taste	7.30	5.46	7.30	5.46	7.26	5.44	7.20	5.40	6.80	5.00	6.75	4.90	6.53	4.83
OAA	7.86	7.20	7.86	7.19	7.84	7.17	7.77	7.13	7.63	6.98	7.55	6.88	7.42	6.78
Total Score	47.24	43.21	47.23	43.14	47.03	43.02	46.73	42.79	45.82	41.86	45.31	41.28	44.54	40.69

4.7.5.2. Organoleptic qualities of fermented and unfermented food mixtures on storage- T_2

Table 85 showed that initially in T_2, the mean score for appearance in FFM and UFFM was found to be 8.36 and 8.30 which decreased on storage and after six months the score was reduced to 8.13 and 8.06 respectively. The colour of FFM and UFFM had a mean score 8.40 which on storage for six months decreased to 8.20 in both FFM and UFFM. The flavour of FFM and UFFM showed a marked difference with 7.13 and 5.76 initially and by the end of the storage period, the mean score for flavour in FFM and UFFM was reduced to 6.93 and 5.27 respectively. The texture of FFM and UFFM was 8.40 and 8.20 initially, which was decreased on storage to 8.03 and 7.86 respectively. With regard to taste, FFM had a score of 7.26 initially which decreased on storage to 6.63. The taste for UFFM was less (5.56) compared to FFM which decreased on storage and after six months the mean score was 4.90. Overall acceptability also showed a decrease, on storage. In FFM, OAA was 7.95 initially which was reduced to 7.58 and in UFFM the initial mean score of 7.24 was reduced to 6.85 after six months of storage

4.7.5.3. Organoleptic qualities of fermented and unfermented food mixtures on storage- T_3

As in Table 86, For the food mixture T_3, the mean score for appearance of FFM and UFFM was 8.33 and 8.20 initially which was reduced with storage and after six months the score was 8.10 and 7.89 respectively. Initially colour of FFM and UFFM had a mean score 8.56 and 8.53 which on storage for six months decreased to 8.36 and 8.28. The mean score for flavour of FFM and UFFM recorded a difference with 7.10 and 5.78 initially, and in both FFM and UFFM, there was a reduction in the flavour. By the end of the storage period, the mean score of flavour in FFM and UFFM was 6.61 and 5.30 respectively. The texture of FFM and UFFM was 8.31 and 8.20 initially and this was decreased on storage to 8.00 and 7.68 respectively after six months. With regard to taste, FFM had a score of 7.53 initially which decreased on storage to 6.87. The mean score for taste of UFFM was 5.70 which decreased on storage and after six months the mean score was 5.05.The mean score for OAA of FFM which was 7.97

Table 85. Mean score for organoleptic qualities of fermented and unfermented food mixture on storage.-T2

Quality attributes	Storage period in months													
	Initial		1		2		3		4		5		6	
	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM
Appearance	8.36	8.30	8.36	8.30	8.35	8.30	8.30	8.26	8.23	8.21	8.16	8.11	8.13	8.06
Colour	8.40	8.40	8.40	8.40	8.38	8.38	8.33	8.34	8.30	8.30	8.26	8.24	8.20	8.20
Flavour	7.33	5.76	7.32	5.76	7.27	5.65	7.21	5.60	7.11	5.46	7.00	5.35	6.93	5.27
Texture	8.40	8.20	8.40	8.20	8.40	8.19	8.36	8.17	8.30	8.06	8.23	8.00	8.03	7.86
Taste	7.26	5.56	7.25	5.56	7.23	5.55	7.16	5.50	6.83	5.13	6.76	5.00	6.63	4.90
OAA	7.95	7.24	7.95	7.24	7.92	7.21	7.87	7.17	7.75	7.03	7.68	6.94	7.58	6.85
Total Score	47.7	43.464	47.676	43.464	47.556	43.284	47.232	43.044	46.524	42.192	46.092	41.64	45.504	41.148

.

Table 86. Mean score for organoleptic qualities of fermented and unfermented food mixture on storage.-T3

Quality attributes	Storage period in months													
	Initial		1		2		3		4		5		6	
	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM
Appearance	8.33	8.20	8.33	8.20	8.31	8.19	8.27	8.16	8.20	8.10	8.15	8.03	8.10	7.89
Colour	8.56	8.53	8.55	8.53	8.53	8.50	8.48	8.45	8.44	8.40	8.40	8.33	8.36	8.28
Flavour	7.10	5.78	7.10	5.77	7.03	5.67	6.86	5.63	6.80	5.50	6.70	5.38	6.61	5.30
Texture	8.31	8.20	8.31	8.20	8.29	8.20	8.27	8.15	8.16	8.00	8.06	7.89	8.00	7.68
Taste	7.53	5.70	7.53	5.69	7.50	5.69	7.43	5.65	7.13	5.24	6.99	5.17	6.87	5.05
OAA	7.97	7.28	7.96	7.28	7.93	7.25	7.86	7.21	7.75	7.05	7.66	6.96	7.59	6.84
Total Score	47.796	43.692	47.784	43.668	47.592	43.5	47.172	43.248	46.476	42.288	45.96	41.76	45.528	41.04

initially was reduced to 7.59 and that of UFFM, which was 7.28 to 6.84 after storage for six months.

4.7.5.4. Organoleptic qualities of fermented and unfermented food mixtures on storage- T_7

As revealed in Table 87, for the food mixture T_7, the initial mean score for appearance of FFM and UFFM was 8.20 and 8.33 which was reduced with storage time and after six months the score was 7.83 and 8.00 respectively. There was no difference in the mean score for colour in FFM and UFFM initially (8.56) but there was a decrease to 8.30 in both FFM and UFFM on storage for six months. The mean score for flavour of FFM and UFFM recorded a difference with 7.00 and 5.80 initially, and in both FFM and UFFM, there was a reduction in the flavour during storage, and after six months, this was 6.68 and 5.29 respectively. The texture of FFM and UFFM was 8.43 and 8.23 initially, and this decreased on storage to 8.08 and 7.84 respectively after six months. With regard to taste, FFM had a score of 7.30 initially which decreased on storage to 6.70. The mean score for taste of UFFM was (5.56) which decreased on storage and after six months the mean score was 4.93. OAA also showed a reduction in mean score after storage. Initially OAA of FFM which was 7.90 was reduced to 7.52 and that of UFFM, 7.30 to 6.87 after six months storage

4.7.5.5. Organoleptic qualities of fermented and unfermented food mixtures on storage- T_8

Initially in T_8 (Table 88) the initial mean score for appearance of FFM and UFFM was 8.43 and 8.40 which decreased on storage and after six months the score was 8.16 and 8.13 respectively. The colour of FFM and UFFM had a mean score 8.40 initially, which on storage for six months decreased to 8.09 and 8.19 respectively. The mean score for flavour of FFM and UFFM showed a marked difference with 7.00 and 5.53 initially and in both FFM and UFFM, there was a reduction in the flavour. By the end of the storage period, the mean score for flavour in FFM and UFFM was 6.58 and 5.07 respectively. The texture of FFM and UFFM was 8.26 and 8.40 initially, and this decreased on storage to 7.93 and 8.10 respectively. With regard to taste FFM had a

Table 87. Mean score for organoleptic qualities of fermented and unfermented food mixture on storage.-T7

Quality attributes	Storage period in months													
	Initial		1		2		3		4		5		6	
	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM
Appearance	8.20	8.33	8.19	8.33	8.18	8.31	8.12	8.26	8.00	8.21	7.88	8.13	7.83	8.00
Colour	8.56	8.56	8.55	8.56	8.52	8.55	8.46	8.46	8.40	8.38	8.35	8.33	8.30	8.30
Flavour	7.00	5.80	7.00	5.78	6.96	5.67	6.91	5.63	6.80	5.50	6.73	5.36	6.68	5.29
Texture	8.43	8.23	8.41	8.23	8.41	8.20	8.37	8.17	8.33	8.03	8.26	8.00	8.08	7.84
Taste	7.30	5.56	7.30	5.55	7.27	5.55	7.20	5.50	6.96	5.11	6.83	5.00	6.70	4.93
OAA	7.90	7.30	7.89	7.29	7.87	7.26	7.81	7.20	7.69	7.05	7.61	6.96	7.52	6.87
Total Score	47.388	43.776	47.34	43.74	47.208	43.536	46.872	43.224	46.188	42.276	45.66	41.784	45.108	41.232

.

.

Table 88. Mean score for organoleptic qualities of fermented and unfermented food mixture on storage.-T8

Quality attributes	Storage period in months													
	Initial		1		2		3		4		5		6	
	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM
Appearance	8.43	8.40	8.40	8.38	8.38	8.38	8.33	8.33	8.25	8.28	8.18	8.18	8.16	8.13
Colour	8.40	8.40	8.38	8.40	8.35	8.37	8.30	8.34	8.25	8.28	8.14	8.23	8.09	8.19
Flavour	7.00	5.53	7.00	5.52	6.93	5.45	6.82	5.39	6.77	5.25	6.65	5.15	6.58	5.07
Texture	8.26	8.40	8.25	8.38	8.22	8.38	8.16	8.35	8.13	8.22	8.06	8.15	7.93	8.10
Taste	7.40	5.80	7.38	5.79	7.35	5.79	7.27	5.73	7.00	5.39	6.87	5.21	6.77	5.14
OAA	7.90	7.31	7.88	7.29	7.85	7.27	7.78	7.23	7.68	7.08	7.58	6.98	7.51	6.93
Total Score	47.388	43.836	47.292	43.764	47.076	43.644	46.656	43.368	46.08	42.504	45.48	41.904	45.036	41.556

score of 7.40 initially which decreased on storage to 6.77. The mean score for taste of UFFM was 5.80 which decreased on storage and after six months, the mean score was 5.14. There was a reduction in the mean score for OAA. OAA of FFM was 7.90 initially, which was reduced to 7.51 and that of UFFM, 7.31 to 6.93 after six months of storage.

4.7.5.6. Organoleptic qualities of fermented and unfermented food mixtures on storage- T_9

Table 89 showed that in T_9, the initial mean score for appearance of FFM and UFFM was 8.43 and 8.36 which decreased on storage, and after six months the score was 8.16 and 8.07 respectively. The colour of FFM and UFFM had a mean score 8.53 and 8.50, which on storage for six months, decreased to 8.30 and 8.28. The mean score for flavour of FFM and UFFM showed a marked difference with 7.20 and 5.83 initially and in both FFM and UFFM, there was a reduction in the flavour. By the end of the storage period, the mean score for flavour content in FFM and UFFM was 6.78 and 5.25 respectively. The score for texture of FFM and UFFM was 8.27 and 8.18 initially, and this decreased on storage to 7.98 and 7.86 respectively. With regard to taste, FFM had a score of 7.40 initially which decreased on storage to 6.77. The mean score for taste of UFFM was 5.50 initially, which decreased on storage and after six months, the mean score was 4.81. The OAA of FFM which was 7.97 initially was reduced to 7.60 and that of UFFM, 7.27 to 6.85 after six months of storage

The overall acceptability of the six FFM on storage is depicted in Fig 27.

4.8 . Selection of three food mixtures

Based on the nutrient composition, acceptability and presence of viable count of *L. acidophilus* in the six FFM during storage, three food mixtures with maximum quality attributes were selected by applying geometric mean scores and is presented in Table 90.

Table 89. Mean score for organoleptic qualities of fermented and unfermented food mixture on storage.-T9

Quality attributes	Storage period in months													
	Initial		1		2		3		4		5		6	
	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM	FFM	UFFM
Appearance	8.43	8.36	8.42	8.36	8.41	8.34	8.35	8.30	8.26	8.25	8.20	8.15	8.16	8.07
Colour	8.53	8.50	8.52	8.49	8.49	8.48	8.44	8.44	8.40	8.40	8.35	8.33	8.30	8.28
Flavour	7.20	5.83	7.18	5.82	7.13	5.72	7.06	5.66	6.93	5.40	6.85	5.30	6.78	5.25
Texture	8.27	8.18	8.27	8.18	8.25	8.16	8.20	8.14	8.16	8.06	8.10	8.00	7.98	7.86
Taste	7.40	5.50	7.40	5.50	7.36	5.48	7.29	5.42	7.00	5.07	6.87	4.92	6.77	4.81
OAA	7.97	7.27	7.96	7.27	7.93	7.24	7.87	7.19	7.75	7.04	7.67	6.94	7.60	6.85
Total Score	47.796	43.644	47.748	43.62	47.568	43.416	47.208	43.152	46.5	42.216	46.044	41.64	45.588	41.124

Table 90. Three fermented food mixtures with maximum quality attributes

Treatment with composition		Geometric mean scores
T_1	B+ DS+M	**11.898**
T_2	B+ DS+P	10.761
T_3	B+ DS+T	**11.931**
T_7	B+ DS+M+P	11.092
T_8	B+ DS+M+T	**11.397**
T_9	B+ DS+P+T	10.788

Highest geometric mean score was for T_3 (11.931) followed by T_1 (11.898) and T_8 (11.397). Thus T_3, T_1 and T_8 can be considered as good probiotic fermented food mixtures with acceptable qualities. These three food mixtures were selected for further modification studies.

4.9. Modifications in the composition of the fermented food mixtures

4.9.1. Standardising the level of added ingredients in the food mixtures.

In the selected three food mixtures**,** substrate composition was modified by the addition of sucrose, skimmed milk powder, wheat bran and sorbitol**.**

4.9.1.1. Standardising the level of sucrose in the food mixtures with maximum viable counts of *L. acidophilus*

Sucrose was added at 5, 10 and 15 percent level in each of the selected three food mixtures before fermentation. Viable counts of *L.acidophilus* were then enumerated after freeze drying and the results are presented n Table 91.

Table 91. Viable count of *L.acidophilus* in the food mixtures with sucrose

Treatments	Viable count (x 10^7 cfu /g)			
	5 % sucrose	10 % sucrose	15% sucrose	FC (without sucrose)
T_1 S	258	241	201	T_1 147
T_3 S	351	322	306	T_3 282
T_8 S	297	254	232	T_8 210

T_1S- Sucrose in T_1, T_3 S- Sucrose in T_3, T_8S- Sucrose in T_8,
Values are mean of three independent enumerations.

Addition of sucrose at 5 per cent level in T_1 S showed a maximum viable count of *L. acidophilus* 258 cfu /g (x 10^7) as against a viable count of 147 x 10^7 cfu/g in FC sample of T_1 without sucrose. Similarly at 5 percent level, T_3S and T_8S showed a maximum viable count of *L .acidophilus* 351 and 297 x 10^7 cfu /g respectively as against a viable count of 282 and 210 x 10^7 cfu/g in the FC samples of T_3 and T_8.

At 10 per cent and 15 per cent level of sucrose, T_1S showed a viable count of 241 and 201 x 10^7 cfu /g respectively as against a viable count of 147 x 10^7 cfu /g in the FC of T_1. Addition of sucrose at 10 per cent and 15 per cent level, T_3S showed a viable count of 322 and 306 x 10^7 cfu /g respectively as against a viable count of 282 x 10^7 cfu /g in the FC of T_3. Addition of sucrose at 10 per cent and 15 per cent level, T_8S showed a maximum viability of 254 and 232 x 10^7 cfu /g respectively as against a viable count of 210 x 10^7 cfu /g in the FC of T_8.

Thus addition of sucrose at 5 per cent, 10 per cent and 15 percent levels to T_1, T_3 and T_8 showed a maximum viable count of *L. acidophilus* in T_3 at 5 per cent level which was selected for further storage studies.

4.9.1.2. Standardising the level of sorbitol in the food mixtures with maximum viable counts of *L. acidophilus.*

Sorbitol was added at 5, 10 and 15 percent level in each of the selected three food mixtures before fermentation. Viable counts of *L .acidophilus* were then enumerated after freeze drying and the results are presented in Table 92**.**

Table 92. Viable count of *L. acidophilus* in the food mixtures with sorbitol

Treatments	Viable count (x 10^7 cfu /g)			
	5 % sorbitol	10 % sorbitol	15% sorbitol	FC (without sorbitol)
T_1 SB	148	146	147	T_1 147
T_3 SB	284	282	280	T_3 282
T_8 SB	211	210	208	T_8 210

T_1SB-Sorbitol in T_1, T_3SB- Sorbitol in T_3, T_8SB-Sorbitol in T_8.
Values are mean of three independent enumerations.

Addition of sorbitol at 5, 10 and 15 percent level did not show much change in the viable count of *L. acidophilus* from the FC of T_1, T_3 and T_8. Maximum viability of *L. acidophilus* was shown by T_3SB (284 x 10^7 cfu/g) at 5 per cent level as against the viable count of FC of T_3 (282 x 10^7 cfu /g). Hence T_3SB at 5 per cent level was selected for further studies.

4.9.1.3. Standardising the level of wheat bran in food mixtures with maximum viable count of *L. acidophilus.*

Wheat bran was added at 5, 10 and 15 percent level in each of the selected three food mixtures before fermentation. Viable counts of *L. acidophilus* were then enumerated after freeze drying and the results are presented in Table 93**.**

Table 93. Viable count of *L. acidophilus* in food mixtures with wheat bran

Treatments	Viable count (x 10^7 cfu /g)			
	5 % Wheat bran	10 % Wheat bran	15% Wheat bran	FC (without Wheat bran)
T_1 W	246	231	194	T_1 147
T_3 W	333	321	300	T_3 282
T_8 W	277	235	221	T_8 210

T_1W- Wheat bran in T_1, T_3W-Wheat bran in T_3, T_8W- Wheat bran in T_8.
Values are mean of three independent enumerations

Addition of wheat bran at 5 percent level in T_1 showed a maximum viable count of *L .acidophilus* 246 x 10^7 cfu/g as against a viable count of 147 x 10^7 cfu/g in the FC of T_1 without wheat bran. Similarly addition of wheat bran at 5 percent level in T_3 and T_8 showed a maximum viable count of *L. acidophilus*, 333 x 10^7 cfu/g and 277 x 10^7 cfu /g as against a viable count of 282 and 210 x 10^7 cfu/g respectively in the FC of T_3 and T_8.

Addition of wheat bran at 10 percent level in T_1, showed a viable count of *L. acidophilus* 231 x 10^7 cfu/g as against a viable count of 147 x 10^7 cfu/g in the FC of T_1. Similarly addition of wheat bran at 10 percent level in T_3 and T_8 showed a viable

count of *L. acidophilus* 231 x 10^7 cfu/g and 235 x 10^7 cfu /g respectively as against a viable count of 282 and 210 x 10^7 cfu/g respectively in the FC of T_3 and T_8.

Addition of wheat bran at 15 percent level in T_1 showed a viable count of *L. acidophilus* 194 x 10^7 cfu/g as against a viable count of 147 x 10^7 cfu/g in the FC.

of T_1. Similarly addition of wheat bran at 15 percent level in T_3 and T_8 showed a viable count of *L. acidophilus* 300 x 10^7 cfu/g and 231 x 10^7 cfu /g as against a viable count of 282 and 210 x 10^7 cfu/g respectively in the FC of T_3 and T_8.

Thus addition of wheat bran at 5 per cent, 10 per cent and 15 percent levels to T_1, T_3 and T_8 showed a maximum viable count of *L. acidophilus* in T_3 at 5 per cent level. Hence T_3W at 5 per cent level was selected for further storage studies.

4.9.1.4. Standardising the level of skimmed milk powder in food mixtures with maximum viable count of *L. acidophilus.*

Skimmed milk powder was added at 5, 10 and 15 percent level in each of the selected three food mixtures before fermentation. Viable counts of *L. acidophilus* were then enumerated after freeze drying and the results are presented in Table 94**.**

Table 94. Viable count of *L .acidophilus* in food mixtures with skimmed milk powder

Treatments	Viable count (x 10^7 cfu /g)			
	5 % Skimmed milk powder	10 % Skimmed milk powder	15% Skimmed milk powder	FC (without skimmed milk powder)
T_1 SK	255	241	204	T_1 147
T_3 SK	347	319	305	T_3 282
T_8 SK	289	234	220	T_8 210

T_1SK- Skimmed milk powder in T_1, T_3SK- Skimmed milk powder in T_3, T_8SK- Skimmed milk powder in T_8.
Values are mean of three independent enumerations.

Addition of skimmed milk powder at 5 percent level in T_1 showed the maximum viable count of *L. acidophilus* 255 x 10^7 cfu/g as against a viable count of 147 x 10^7

cfu/g in the FC of T_1. Similarly addition of skimmed milk powder at 5 percent level in T_3 and T_8 showed a maximum viable count of *L. acidophilus* 347 x 10^7 cfu/g and 289 x 10^7cfu /g respectively as against a viable count of 282 and 210 x 10^7 cfu/g respectively in the FC of T_3 and T_8.

Addition of skimmed milk powder at 10 percent level in T_1 showed a viable count of *L. acidophilus* 241 x 10^7 cfu/g as against a viable count of 147 x 10^7 cfu/g in the FC of T_1. Similarly addition of skimmed milk powder at 10 percent level in T_3 and T_8 showed a viable count of *L. acidophilus* 319 x 10^7 cfu/g and 234 x 10^7 cfu /g respectively as against a viable count of 282 and 210 x 10^7 cfu/g respectively in the FC of T_3 and T_8.

Addition of skimmed milk powder at 15 percent level in T_1 showed a viable count of *L. acidophilus* 204 x 10^7 cfu/g as against a viable count of 147 x 10^7 cfu/g in the FC of T_1. Similarly addition of skimmed milk powder at 15 percent level in T_3 and T_8 showed a viable count of *L. acidophilus* 305 x 10^7 cfu/g and 220 x 10^7 cfu /g respectively as against a viable count of 282 and 210 x 10^7 cfu/g respectively in the FC of T_3 and T_8.

Thus addition of skimmed milk powder at 5 per cent, 10 per cent and 15 percent levels to T_1, T_3 and T_8 showed a maximum viable count of *L. acidophilus* in T_3SK at 5 per cent level.

Maximum viability of *L .acidophilus* was observed in T_3S, T_3SB, T_3W and T3SK at 5 per cent level which was selected for further storage studies.

4.9.2 Storage studies of the selected modified food mixtures.

The selected modified food mixtures i.e. T_3S, T_3SB, T_3W and T_3SK along with the FC of T_3 were packed in metalised polyester/ polyethylene laminated pouches and were stored for a period of six months under ambient conditions .Quality evaluation of these selected food mixtures were done each month for a period of six months.

4.9.2.1. Chemical constituents in modified food mixtures

4.9.2.1.1. Chemical constituents in sucrose added fermented food mixtures.

Moisture

Table 95.Moisture content of sucrose added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	1.831 [a]	1.831 [a]	1.900 [b]	2.135 [c]	2.238 [d]	2.539 [e]	2.971 [f]
FC	1.837 [a]	1.843 [a]	1.903 [b]	2.140 [c]	2.243 [d]	2.547 [e]	2.980 [f]
Mean difference	-0.006	-0.012	-0.003	-0.005	-0.005	-0.008	-0.009
t value	-1.791	-3.781	-1.414	-0.922	-1.493	-3.582	-1.499
Significance	0.148	0.213	0.019	0.230	0.409	0.203	0.208
	NS	NS	NS	NS	NS	NS	NS

T_3S – Sucrose in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

The initial moisture content in T_3S (1.831 g /100g) showed no significant difference upto one month of storage but increased significantly throughout the storage period and reached a maximum of 2.971 g/ 100g by the end of sixth month. But there was no significant difference in the moisture content of T_3S and FC throughout the storage period.

Titrable acidity

Table 96. Titrable acidity in sucrose added fermented food mixture and fermented control on storage (g lactic acid/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	4.021 [a]	3.028 [b]	3.037 [c]	3.154 [d]	3.230 [e]	4.341 [f]	4.579 [g]
FC	2.537 [a]	2.607 [b]	2.653 [c]	2.773 [d]	3.027 [e]	3.250 [f]	3.367 [g]
Mean difference	1.484	0.421	0.384	0.381	0.203	1.091	1.212
t value	445.00	89.095	81.317	40.305	26.833	188.794	256.680
Significance	S	S	S	S	S	S	S

T_3 S – Sucrose in T_3,Values having different super script differ significantly at 5% level DMRT row wise comparison

In T_3S titrable acidity was significantly increasing with the storage period. Initially, titrable acidity was 4.021 g lactic acid /100 g and by the end of the storage period it reached to a maximum of 4.579 g lactic acid /100 g as against 3.367 g lactic acid /100g in FC. There was a significant increase in titrable acidity of T_3S when compared to FC throughout the storage period.

The tirtrable acidity of T_3S (Sucrose in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 27 for comparison.

Starch

Table 97. Starch content in sucrose added fermented food mixture and fermented control on storage on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	54.573 [a]	53.962 [b]	52.870[c]	52.190 [d]	50.373[e]	49.191[f]	47.890[g]
FC	54.567 [a]	53.953 [b]	52.850[c]	52.163 [d]	50.363[e]	49.183[f]	47.853[g]
Mean difference	0.006	0.009	0.02	0.027	0.01	0.008	0.037
t value	0.717	1.020	1.400	1.871	2.50	0.505	2.384
Significance	0.513	0.365	0.234	0.135	0.068	0.640	0.076
	NS	NS	NS	NS	NS	NS	NS

T_3S – Sucrose in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

The starch content in T_3S showed a significant decrease with the storage period. Initially the starch content in T_3S was 54.573g/100g which decreased to 47.890 g /100g by the end of the sixth month. The starch content of T_3S and FC showed no significant difference through out the storage period

Total soluble solids (TSS)

Table 98.Total soluble solids (TSS) in sucrose added fermented food mixture and fermented control on storage (°Brix)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	12.06 [a]	12.08 [a]	12.17 [b]	12.18 [b]	12.22 [c]	12.27 [c]	12.34 [d]
FC	11.65 [a]	11.67 [a]	11.68 [b]	11.70 [b]	11.73 [c]	11.75 [c]	11.79 [d]
Mean difference	0.41	0.41	0.49	0.48	0.49	0.52	0.55
t value	53.66	56.34	47.12	50.55	101.82	57.45	50.91
Significance	S	S	S	S	S	S	S

T_3S – Sucrose in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

There was no significant difference in the TSS content of T_3S upto one month of storage. Initially TSS was 12.06°brix and by the end of sixth month TSS was 12.34°brix.There was a significant increase in the TSS content in T_3S than FC throughout the storage period.

The TSS of T_3S (Sucrose in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 28 for comparison.

Reducing sugars and total sugars

Table 99 .Reducing sugar in sucrose added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	3.77 [e]	3.82 [de]	3.87 [d]	3.90 [cd]	3.92 [c]	3.96 [b]	4.40 [a]
FC	2.76 [f]	2.77 [ef]	2.79 [de]	2.81 [cd]	2.83 [bc]	2.85 [b]	2.87 [a]
Mean difference	1.01	1.05	1.08	1.09	1.09	1.11	1.53
t value	72.76	109.24	112.43	113.84	86.32	82.00	105.94
Significance	S	S	S	S	S	S	S

T_3 S – Sucrose in T_3,Values having different super script differ significantly at 5% level DMRT row wise comparison

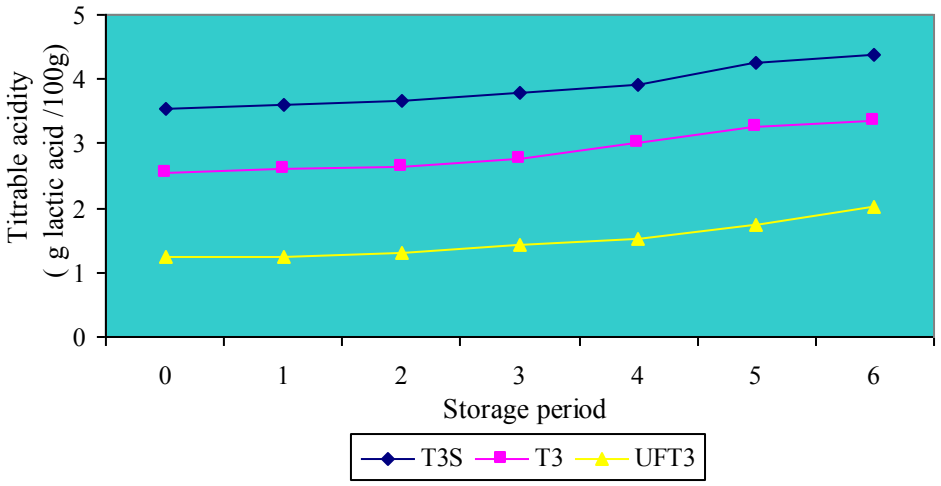


Fig .27. Titrable acidity of T3S, T3 and UFT3

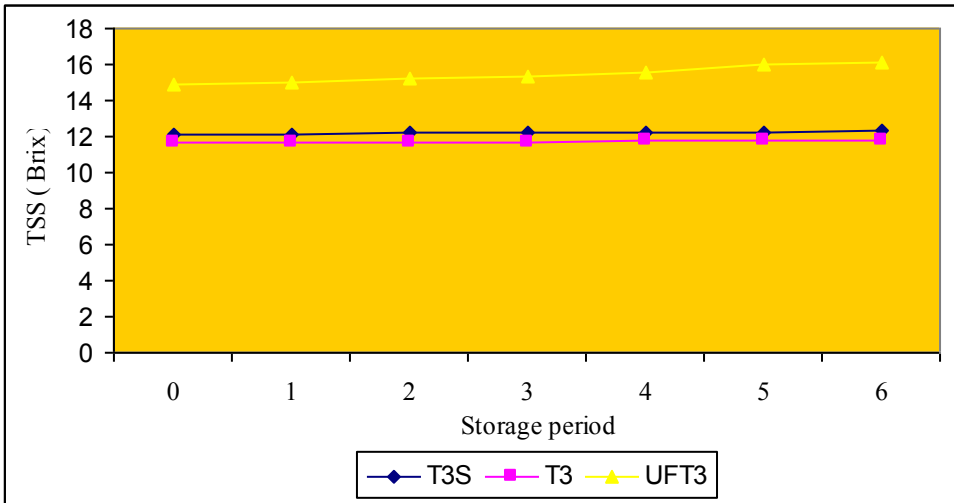


Fig.28.TSS of T3S, T3 and UFT3

Initially reducing sugar was 3.77 g/ 100 g in T_3S where as in FC it was 2.76 g/100g. There was no significant difference in the reducing sugar content of T_3S upto two months of storage. After two months, the reducing sugar content increased significantly and by the end of the sixth month, it was 4.40 g/100g as against 2.87 g/100g in FC. There was a significant increase in the reducing sugar content in T_3S compared to FC throughout the storage period.

The tirtrable acidity of T_3S (Sucrose in T_3), T_3 and UFT_3(unfermented T_3) is depicted in Fig 29 for comparison.

Total sugar

Table 100. Total sugar in sucrose added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	6.28 ^{f}	6.54 ^{e}	6.56 ^{d}	6.56 ^{d}	6.58 ^{c}	6.65 ^{b}	6.72 ^{a}
FC	5.25 ^{f}	5.24 ^{f}	5.26 ^{e}	5.27 ^{d}	5.59 ^{c}	5.32 ^{b}	5.35 ^{a}
Mean difference	1.03	1.3	1.3	1.29	0.99	1.33	1.37
t value	116.41	165.02	75.48	89.75	191.50	89.21	91.23
Significance	S	S	S	S	S	S	S

T_3S – Sucrose in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

Initial total sugar in T_3S was 6.28 as against 5.25 g/100g in FC. The total sugar in T_3S increased significantly with storage. There was no significant difference in the total sugar content in the second and third month of storage. By the end of the sixth month, total sugar content in T_3S significantly increased to 6.72 g /100 g whereas in FC it was 5.35g/100g. There was a significant increase in the total sugar content in T_3S compared to FC throughout the storage period.

The total sugar of T_3S (Sucrose in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 30 for comparison.

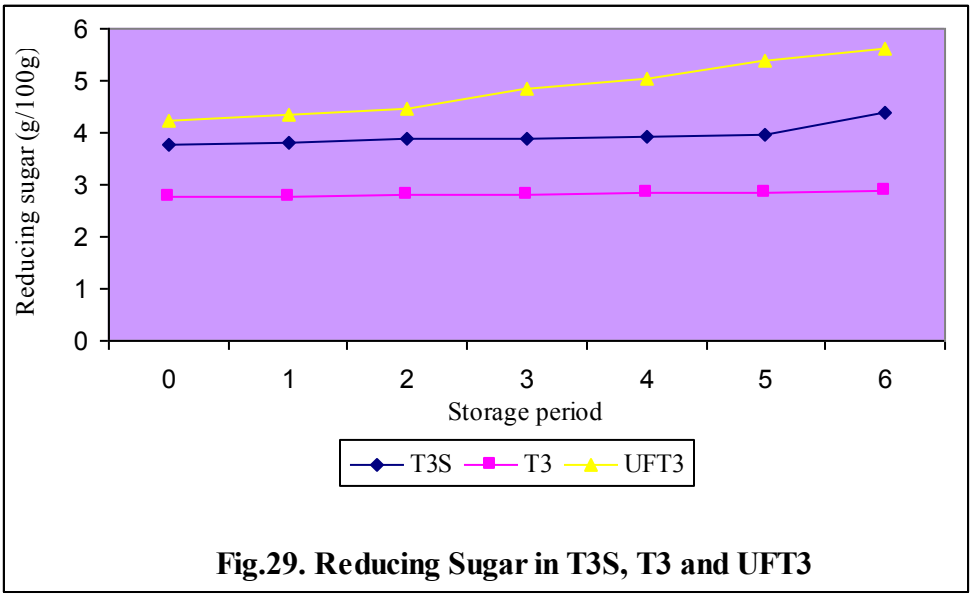


Fig.29. Reducing Sugar in T3S, T3 and UFT3

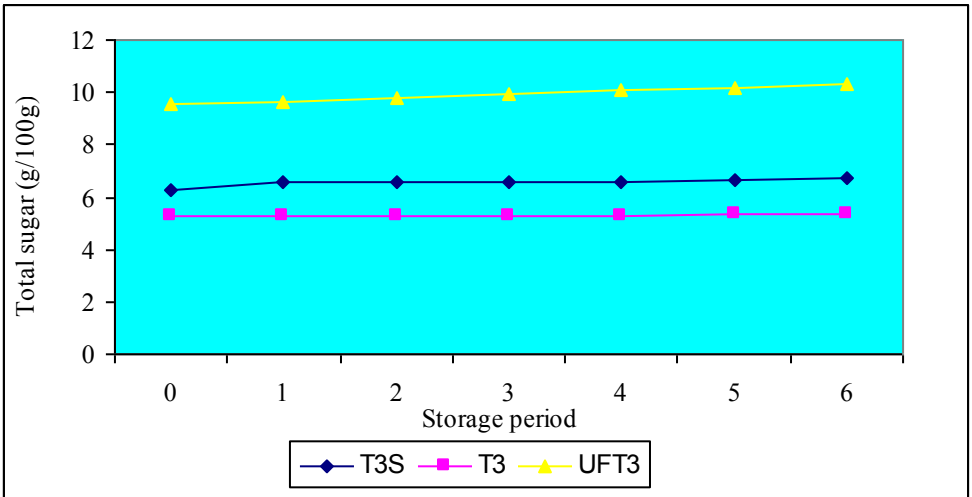


Fig .30. Total Sugar in T3S, T3 and UFT3

Protein

Table 101. Protein content in sucrose added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	9.830 [a]	9.670 [b]	9.460 [c]	9.066 [d]	8.665 [e]	8.440 [f]	8.240 [g]
FC	9.805 [a]	9.670 [b]	9.450 [c]	9.063 [d]	8.663 [e]	8.433 [f]	8.243 [g]
Mean difference	0.025	0.0001	0.01	0.003	0.002	0.007	-0.003
t value	1.342	0.316	0.243	0.894	0.850	0.354	-0.707
Significance	0.251	0.768	0.820	0.422	0.512	0.742	0.519
	NS	NS	NS	NS	NS	NS	NS

T_3S – Sucrose in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

Initially the protein content in T_3S was 9.830 g/100g as against 9.805 g/100g in FC. Protein content decreased significantly with storage in both samples and by the end of the sixth month, the protein content was 8.240 g/100g in T_3S.There was no significant difference in the protein content of T_3S and FC throughout the storage period.

β carotene

Table 102. β carotene content in sucrose added fermented food mixture and fermented control on storage (µg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	470.93 [a]	467.84 [b]	463.15[c]	455.23 [d]	429.03[e]	402.14[f]	377.19[g]
FC	470.91 [a]	467.84 [b]	463.16[c]	455.24 [d]	429.04[e]	402.17[f]	377.16[g]
Mean difference	0.02	0.0001	-0.01	-0.01	-0.01	-0.03	0.03
t value	1.789	0.0001	-0.905	-0.447	-1.213	-3.162	2.183
Significance	0.173	1.000	0.420	0.686	0.337	0.064	0.147
	NS	NS	NS	NS	NS	NS	NS

T_3S – Sucrose in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

Initially, the β carotene content in T_3S was 470.93 µg/100g and in FC it was 470.91 µg /100g. β carotene decreased significantly in T_3S and FC on storage, and by the end of the sixth month β carotene content in T_3S was significantly reduced to 377.19

µg/100g. There was no significant difference in the β carotene content of T_3S and FC throughout the storage period.

Calcium

Table 103.Calcium content in sucrose added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	69.700 [a]	69.376[b]	68.416[c]	66.136[c]	63.860[e]	62.226[f]	60.936 [g]
FC	69.700 [a]	69.373[b]	68.411 [c]	66.120 [c]	63.870 [e]	62.203 [f]	60.960[g]
Mean difference	0.0001	0.003	0.005	0.016	-0.01	0.023	-0.024
t value	0.0001	0.186	0.0001	1.581	-0.548	2.275	-1.323
Significance	1.00	0.862	1.00	0.189	0.613	0.070	0.256
	NS	NS	NS	NS	NS	NS	NS

T_3 S – Sucrose in T_3,Values having different super script differ significantly at 5% level DMRT row wise comparison

Initially there was no difference in calcium content of T_3S (69.700 mg/100g) and FC. The calcium content in T_3S and FC decreased significantly on storage. There was no significant difference in the calcium content of T_3S and FC throughout the storage period.

Potassium

Table 104. Potassium content in sucrose added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	396.663[a]	396.260[b]	396.040[c]	393.043[d]	392.156[e]	389.950[f]	377.810[g]
FC	396.670[a]	396.263[b]	396.040[c]	393.046[d]	392.153[e]	389.946[f]	377.823[g]
Mean difference	-0.007	-0.003	0.0001	-0.003	0.003	0.004	-0.013
t value	0.213	-0.171	1.00	-0.177	-0.267	-0.250	-1.265
Significance	0.842	0.872	0.374	0.868	0.802	0.815	0.275
	NS	NS	NS	NS	NS	NS	NS

T_3S – Sucrose in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

Initially, the potassium content in T_3S was 396.663 mg/100g and in FC it was 396.670 mg/100g. Potassium content decreased significantly on storage in T_3S and FC,

and by the end of the sixth month, the potassium content was significantly reduced to 377.810 mg/100g in T_3S and to 377.823 mg/100g in FC. There was no significant difference in the potassium content of T_3S and FC throughout the storage period.

Iron

Table 105. Iron content in sucrose added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	6.76 [a]	6.54 [b]	6.35 [c]	6.14 [d]	6.02 [e]	5.86 [f]	5.65[g]
FC	6.79 [a]	6.55 [b]	6.37 [c]	6.17 [d]	6.06 [e]	5.87 [f]	5.66[g]
Mean difference	-0.03	-0.01	-0.02	-0.03	-0.04	-0.01	-0.01
t value	-0.898	-0.970	-1.750	-1.387	1.00	-1.00	-1.30
Significance	0.420	0.387	0.155	0.238	0.345	0.374	0.263
	NS	NS	NS	NS	NS	NS	NS

T_3S – Sucrose in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

Initially, iron content in T_3S was 6.76 mg/100g and in FC it was 6.79 mg/100g. There was a significant decrease in the iron content in T_3S and FC with storage. By the end of the sixth month, the iron content was significantly reduced to 5.65 mg/100g in T_3S. There was no significant difference in the iron content of T_3S and FC throughout the storage period.

Thiamine

Table 106. Thiamine content in sucrose added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	0.099[a]	0.088 [b]	0.079[c]	0.071[d]	0.058[e]	0.052[f]	0.044[g]
FC	0.089[a]	0.069 [b]	0.058[c]	0.051[d]	0.044[e]	0.029[f]	0.023[g]
Mean difference	0.010	0.019	0.021	0.02	0.014	0.023	0.021
t value	50.50	90.09	44.54	52.42	80.78	35.00	6.18
Significance	S	S	S	S	S	S	S

T_3S – Sucrose in T_3,Values having different super script differ significantly at 5% level DMRT row wise comparison

The thiamine content in T_3S was 0.099 mg/100g initially which reduced significantly to 0.044 mg/100g by the end of the sixth month. Thiamine content in T_3S was significantly high when compared to FC throughout the storage period.

The thiamine content of T_3S (Sucrose in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 31 for comparison.

Riboflavin

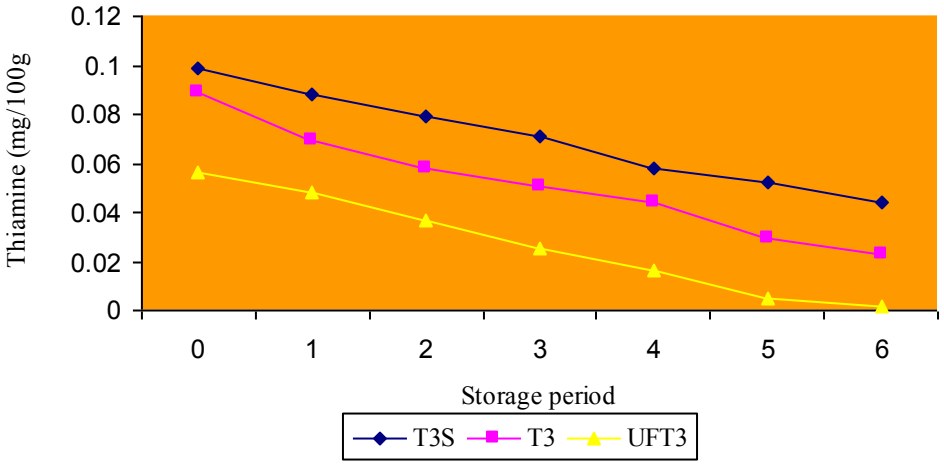
Table 107. Riboflavin content in sucrose added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	0.83^a	0.74^b	0.69^c	0.63^d	0.43^e	0.36^f	0.33^g
FC	0.61^a	0.41^b	0.34^c	0.33^d	0.28^e	0.24^f	0.15^g
Mean difference	0.22	0.33	0.35	0.3	0.15	0.12	0.18
t value	44.54	48.50	72.44	23.78	30.59	30.47	27.50
Significance	S	S	S	S	S	S	S

T_3S – Sucrose in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

The riboflavin content in T_3S was 0.83mg/100g initially which significantly reduced to 0.33 mg/100g by six months of storage. Riboflavin content of T_3S was significantly high than FC throughout the storage period.

The riboflavin content of T_3S (Sucrose in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 32 for comparison.



Fig, 31. Thiamine content of T3S, T3 and UFT3

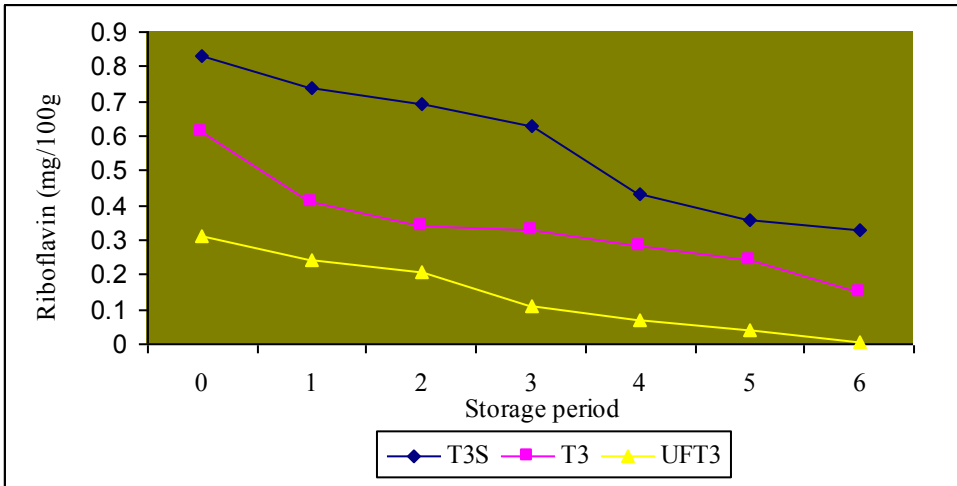


Fig.32. Riboflavin content of T3S, T3 and UFT3

In vitro starch digestibility (IVSD)

Table 108. IVSD of sucrose added fermented food mixture and fermented control on storage (per cent)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	80.766 [f]	80.633 [f]	81.466[e]	81.666 [d]	82.533[c]	82.733[b]	83.66 [a]
FC	80.631 [e]	80.700 [e]	81.202[d]	81.399 [d]	82.333[c]	82.627[b]	83.233[a]
Mean difference	0.135	0.067	0.264	0.267	0.200	0.106	0.427
t value	2.00	0.229	2.219	0.0001	1.809	0.447	2.673
Significance	0.116	0.830	0.091	1.00	0.145	0.678	0.056
	NS	NS	NS	NS	NS	NS	NS

T_3S – Sucrose in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

Initially in T_3S, IVSD was 80.766 per cent and in FC it was 80.631 per cent. IVSD in both T_3S and FC increased significantly on storage and by the end six months, in T_3S it was 83.66 per cent and in FC it was 83.233 per cent. There was no significant difference in the IVSD of T_3S and FC throughout the storage period**.**

In vitro protein digestibility (IVPD)

Table 109. IVPD of sucrose added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3S	85.56 [f]	85.58 [f]	85.86 [e]	86.21 [d]	86.93 [c]	87.13 [b]	87.57 [a]
FC	85.56 [f]	85.58 [f]	85.85 [e]	86.21 [d]	86.94 [c]	87.13 [b]	87.59 [a]
Mean difference	0.0001	0.0001	0.01	0.0001	-0.01	0.0001	-0.02
t value	0.447	0.624	0.340	0.0001	-0.316	-0.447	-0.640
Significance	0.678	0.202	0.751	1.00	0.768	0.678	0.557
	NS	NS	NS	NS	NS	NS	NS

T_3S – Sucrose in T_3,Values having different super script differ significantly at 5% level DMRT row wise comparison

Initially, IVPD in T_3S and FC was 85.56 per cent which increased significantly on storage and by the end of six months the IVPD was 87.57 per cent in T_3S and 87.59 per cent in FC. There was no significant difference in the IVPD of T_3S and FC throughout the storage period.

4.9.2.1.2. Chemical constituents in sorbitol added fermented food mixture on storage

Moisture

Table 110. Moisture content of sorbitol added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	1.833 [a]	1.833 [a]	1.900 [b]	2.137 [c]	2.240 [d]	2.539 [e]	2.974 [f]
FC	1.837 [a]	1.843 [a]	1.903 [b]	2.140 [c]	2.243 [d]	2.547 [e]	2.980 [f]
Mean difference	-0.004	-0.01	-0.003	-0.003	-0.003	-0.008	-0.006
t value	-1.10	-1.34	-2.12	-0.57	-0.36	-2.20	-0.91
Significance	0.333	0.251	0.101	0.595	0.735	0.092	0.413
	NS	NS	NS	NS	NS	NS	NS

T_3SB -Sorbitol in T_3,Values having different super script differ significantly at 5% level DMRT row wise comparison

Initially, the moisture content of T_3SB was 1.833 as against 1.837 in FC. There was no significant difference in the moisture content of T_3SB and FC upto one month of storage but after that increased significantly throughout the storage period. After six months, the moisture content was maximum in FC (2.980 g/100g) as against T_3SB (2.974 g/ 100). But there was no significant difference in the moisture content of T_3SB and FC throughout the storage period.

Titrable acidity

Table.111. Titrable acidity in sorbitol added fermented food mixture and fermented control and storage (g lactic acid/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	2.543 [a]	2.610 [b]	2.650 [c]	2.777 [d]	3.033 [e]	3.250 [f]	3.377 [g]
FC	2.537 [a]	2.607 [b]	2.653 [c]	2.773 [d]	3.027 [e]	3.250 [f]	3.367 [g]
Mean difference	0.006	0.003	-0.003	0.004	0.006	0.0001	0.01
t value	1.990	1.342	0.447	0.453	0.252	-0.500	1.393
Significance	0.117	0.251	0.678	0.674	0.814	0.643	0.236
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

Titrable acidity was found to be higher in T_3SB than FC. Titrable acidity was significantly increasing with the storage period in both FC and T_3SB. Initially titrable acidity was 2.543 g lactic acid /100g in T_3SB and by the end of the storage period; it reached to a maximum of 3.377 g lactic acid /100g whereas titrable acidity of FC was 3.367 g/100g. There was no significant difference in titrable acidity of T_3SB and FC throughout the storage period.

Starch

Table 112. Starch content of sorbitol added fermented food mixture and fermented control and on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	54.560 [a]	53.957 [b]	52.850 [c]	52.147 [d]	50.357 [e]	49.190 [f]	47.860 [g]
FC	54.567 [a]	53.953 [b]	52.850 [c]	52.163 [d]	50.363 [e]	49.183 [f]	47.853 [g]
Mean difference	-0.007	0.004	0.0001	-0.016	-0.006	0.007	0.007
t value	-0.354	-0.429	0.277	-1.322	-1.319	0.224	0.671
Significance	0.742	0.690	0.795	0.257	0.161	0.834	0.539
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

The starch content in T_3SB and FC showed a significant decrease with the storage period. Initially the starch content in T_3SB was 54.560g/100g which decreased

to 47.860 g /100g by the end of six months. The starch content of T_3SB and FC showed no significant difference throughout the storage period

Total soluble solids (TSS)

Table 113. Total soluble solids (TSS) in sorbitol added fermented food mixture and fermented control on storage (°Brix)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	11.65 [a]	11.66 [a]	11.69 [b]	11.71 [b]	11.72 [c]	11.76 [c]	11.79 [d]
FC	11.65 [a]	11.67 [a]	11.68 [b]	11.70 [b]	11.73 [c]	11.75 [c]	11.79 [d]
Mean difference	0.0001	-0.01	0.01	0.01	-0.01	0.01	0.0001
t value	-1.342	0.0001	0.414	-0.707	-1.243	-1.00	-0.632
Significance	0.251	1.00	0.230	0.519	0.263	0.374	0.561
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

There was an increase in TSS content in both T_3SB and FC during storage. Initially TSS in T_3SB and FC was 11.65°brix. There was no significant difference in the TSS content of T_3SB and FC upto one month of storage. By the end of sixth month, TSS was significantly increased to 1.79°brix in both T_3SB and FC. There was no significant difference in the TSS content in T_3SB and FC throughout the storage period.

Reducing sugar and total sugars

Table 114.Reducing sugar in sorbitol added fermented food mixture and fermented control and storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	2.763 [ef]	2.763 [ef]	2.793 [de]	2.810 [cd]	2.833 [bc]	2.843 [b]	2.866 [a]
FC	2.766 [ef]	2.773 [e]	2.796 [de]	2.813 [cd]	2.836 [bc]	2.853 [b]	2.866 [a]
Mean difference	-0.003	-0.01	-0.003	-0.003	-0.003	-0.01	0.0001
t value	-2.207	-2.121	-2.130	-1.414	-0.707	-2.121	0.0001
Significance	0.123	0.101	0.138	0.230	0.519	0.101	1.00
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3,Values having different super script differ significantly at 5% level DMRT row wise comparison

Reducing sugar was found to be slightly higher in T_3SB initially (2.763g/100g). There was no significant difference in the reducing sugar content upto two months of storage in both T_3SB and FC. After two months, the reducing sugar content increased significantly and by the end of sixth month, it was 2.866 g/100g in T_3SB and FC. There was no significant difference in the reducing sugar content in T_3SB and FC throughout the storage period.

Total sugar

Table 115. Total sugar in sorbitol added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	5.250 ^{e}	5.300 ^{cd}	5.270 ^{a}	5.260 ^{e}	5.360 ^{de}	5.320 ^{bc}	5.359 ^{b}
FC	5.250 ^{f}	5.243 ^{f}	5.260 ^{e}	5.270 ^{d}	5.300 ^{c}	5.316 ^{b}	5.354 ^{a}
Mean difference	0.0001	0.057	0.01	-0.01	0.06	0.004	0.005
t value	0.0001	0.756	0.277	-0.802	1.130	0.243	0.594
Significance	1.000	0.492	0.795	0.468	0.125	0.820	0.422
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

Initially, total sugar in T_3SB and FC was 5.250 g/100g. In T_3SB, during the first month a significant increase in total sugar was observed (5.300 g/100g) but this was significantly reduced to 5.270 g/100g by the second month. Maximum total sugar was observed during the fourth month of storage (5.360 g/100g). By the end of the sixth month the reducing sugar content in T_3SB was 5.359 g /100 g. There was no significant difference in the reducing sugar content in T_3SB and FC throughout the storage period.

4.7.1.6. Protein

Table 116. Protein content in sorbitol added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	9.820 [a]	9.662 [b]	9.457 [c]	9.053 [d]	8.653 [e]	8.443 [f]	8.247 [g]
FC	9.805 [a]	9.670 [b]	9.450 [c]	9.063 [d]	8.663 [e]	8.433 [f]	8.243 [g]
Mean difference	0.015	-0.008	0.007	-0.01	-0.01	0.01	0.004
t value	0.894	-1.845	0.524	-1.283	-2.121	1.209	0.755
Significance	0.422	0.139	0.698	0.269	0.101	0.293	0.492
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

Initially the protein content in T_3SB was 9.820 g/100g which decreased significantly with storage and by the end of sixth month the protein content was 8.247 g/100g. There was no significant difference in the protein content of T_3SB and FC throughout the storage period

β carotene

Table 117. β carotene content in sorbitol added fermented food mixture and fermented control on storage (µg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	470.907[a]	467.846[b]	463.166[c]	455.246[d]	429.043[e]	402.183[f]	377.183[g]
FC	470.913[a]	467.840[b]	463.156[c]	455.237[d]	429.043[e]	402.170[f]	377.157[g]
Mean difference	-0.006	0.006	0.01	0.009	0.0001	0.013	0.026
t value	-0.894	0.632	1.342	1.342	0.0001	1.265	1.940
Significance	0.422	0.561	0.251	0.251	1	0.275	0.124
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3,Values having different super script differ significantly at 5% level DMRT row wise comparison

Initially the β carotene content in T_3SB was 470.907 µg/100g which decreased significantly on storage and by the end of the sixth month, β carotene content in T_3SB

was significantly reduced to 377.183 µg/100g. There was no significant difference in the β carotene content of T_3SB and FC throughout the storage period.

Calcium

Table 118.Calcium content in sorbitol added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	69.71 [a]	69.36[b]	68.42 [c]	66.11 [c]	63.88 [e]	62.21 [f]	60.95 [g]
FC	69.70 [a]	69.37[b]	68.41 [c]	66.12 [c]	63.87 [e]	62.20 [f]	60.96[g]
Mean difference	0.01	-0.01	0.01	-0.01	0.01	0.01	-0.01
t value	2.121	-2.121	1.414	-1.000	0.426	2.121	-0.426
Significance	0.101	0.101	0.230	0.374	0.692	0.101	0.692
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3,Values having different super script differ significantly at 5% level DMRT row wise comparison

The calcium content in T_3SB decreased significantly on storage from 69.710 mg/100g initially, to 60.950 mg/100g by the end of storage. There was no significant difference in the calcium content of T_3SB and FC throughout the storage period

Potassium

Table 119. Potassium content of sorbitol added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	396.653[a]	396.256[b]	396.046[c]	393.056[d]	392.163[e]	389.993[f]	377.830[g]
FC	396.670[a]	396.260[b]	396.040[c]	393.046[d]	392.153[e]	389.950[f]	377.823[g]
Mean difference	-0.017	-0.004	0.006	0.01	0.01	0.043	0.007
t value	-1.00	-0.707	1.00	1.061	0.0001	0.354	0.354
Significance	0.374	0.594	0.374	0.349	1.00	0.742	0.742
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3,Values having different super script differ significantly at 5% level DMRT row wise comparison

The potassium content in T_3SB was 396.653 mg/100g initially which decreased significantly on storage, and by the end of the sixth month, the potassium content was 377.830 mg/100g. The potassium content was more in FC than T_3SB upto one month of storage. After one month, there was an increase in the potassium content in T_3SB than FC through out the storage period. There was no significant difference in the potassium content in T_3SB and FC throughout the storage period.

Iron

Table 120. Iron content of sorbitol added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	6.776 [a]	6.546 [b]	6.376 [c]	6.166 [d]	6.055 [e]	5.863 [f]	5.669 [g]
FC	6.790 [a]	6.553 [b]	6.370 [c]	6.171 [d]	6.060 [e]	5.873 [f]	5.661 [g]
Mean difference	-0.014	-0.007	0.006	-0.005	-0.005	-0.01	0.008
t value	-0.500	-1.342	0.632	-0.543	- 1.233	-1.342	0.516
Significance	0.643	0.251	0.561	0.666	0.232	0.251	0.768
	NS	NS	NS	NS	NS	NS	NS

T_3SB – Sorbitol in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant decrease in the iron content in T_3SB and FC with storage. The initial iron content in T_3SB was 6.776 mg/100g and by the end of the sixth month, the iron content was significantly reduced to 5.669 mg/100g. There was no significant difference in iron content of T_3SB and FC throughout the storage period.

Thiamine

Table 121. Thiamine content of sorbitol added fermented food mixture and fermented food control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	0.0873^a	0.0687^b	0.0567^c	0.0523^d	0.0447^e	0.0280^f	0.0190^g
FC	0.0890^a	0.0690^b	0.0577^c	0.0513^d	0.0443^e	0.0287^f	0.0230^g
Mean difference	-0.0017	-0.0003	-0.001	0.001	0.0004	-0.0007	-0.004
T value	-1.00	-1.414	-2.121	2.121	0.447	-2.00	-1.281
Significance	0.374	0.230	0.101	0.101	0.678	-116	0.269
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

The thiamine content in T_3SB was 0.0873 mg/100g initially, which reduced significantly to 0.0190 mg/100g by the end of the sixth month. There was no significant difference in the thiamine content of T_3SB and FC throughout the storage period.

4.7.1.1 Riboflavin

Table 122. Riboflavin content of sorbitol added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	0.620^a	0.426^b	0.340^c	0.333^d	0.286^e	0.236^f	0.146^g
FC	0.610^a	0.416^b	0.343^c	0.326^d	0.280^e	0.240^f	0.150^g
Mean difference	0.01	0.01	-0.003	0.007	0.006	-0.004	-0.004
t value	0.500	2.121	-0.500	0.707	1.00	-0.500	-0.500
Significance	0.643	0.101	0.643	0.519	0.374	0.643	0.643
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3,Values having different super script differ significantly at 5% level DMRT row wise comparison

The riboflavin content in T_3SB was 0.620 mg/100g initially, which was significantly reduced to 0.146 mg/100g by six months of storage. Eventhough FC showed slightly higher value (0.150 mg/100g) after six month, there was no significant

difference in the thiamine content of T_3SB compared to FC throughout the storage period.

***In vitro* starch digestibility (IVSD)**

Table 123. IVSD of sorbitol added fermented food mixture and fermented control on storage (per cent)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	80.666 ^{e}	80.710 ^{e}	81.260^{d}	81.446 ^{d}	82.313^{c}	82.623^{b}	83.266^{a}
FC	80.633 ^{e}	80.700 ^{e}	81.201^{d}	81.400 ^{d}	82.333^{c}	82.630^{b}	83.233^{a}
Mean difference	0.033	0.01	0.059	0.046	-0.02	-0.007	0.033
t value	1.387	0.949	0.599	2.493	-0.346	-1.571	0.354
Significance	0.238	0.396	0.581	0.067	0.747	0.191	0.742
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

The IVSD was 80.666 per cent initially in T_3SB which increased significantly on storage and by the end of six months, the IVSD in T_3SB was 83.266 per cent. There was no significant difference in the IVSD of T_3SB and FC throughout the storage period**.**

4.7.1.1 *In vitro* protein digestibility (IVPD)

Table 124. IVPD of fermented control and sorbitol added fermented food mixtures on storage (per cent)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SB	85.563 ^{f}	85.570 ^{f}	85.836^{e}	86.216 ^{d}	86.916^{c}	87.133^{b}	87.566^{a}
FC	85.556 ^{f}	85.580 ^{f}	85.850^{e}	86.206 ^{d}	86.937^{c}	87.133^{b}	87.586^{a}
Mean difference	0.007	-0.01	-0.014	0.01	-0.021	0.0001	-0.02
t value	1.99	-1.342	-0.447	0.453	-0.252	0.500	-1.393
Significance	0.117	0.251	0.678	0.674	0.814	0.643	0.236
	NS	NS	NS	NS	NS	NS	NS

T_3SB-Sorbitol in T_3, Values having different super script differ significantly at 5% level DMRT row wise comparison

The IVPD in T_3SB was 85.563 per cent initially, which increased significantly on storage, and by the end of six months, the IVPD was significantly increased 87.566 per cent. There was no significant difference in the IVPD of T_3SB and FC throughout the storage period.

4.9.2.1.3. Chemical constituents in wheat bran added fermented food mixture on storage

Moisture

Table 125. Moisture content of wheat bran added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	1.833 [a]	1.842 [a]	1.906 [b]	2.132 [c]	2.230 [d]	2.537 [e]	2.974 [f]
FC	1.837 [a]	1.843 [a]	1.903 [b]	2.140 [c]	2.243 [d]	2.547 [e]	2.980 [f]
Mean difference	-0.004	-0.001	0.003	-0.008	-0.013	-0.01	-0.006
t value	-0.447	-0.383	0.788	-1.231	-2.000	-1.342	-1.034
Significance	0.678	0.721	0.475	0.286	0.116	0.251	0.360
	NS	NS	NS	NS	NS	NS	NS

T_3W-Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

The moisture content showed no significant difference upto one month of storage but increased significantly throughout the storage period and reached a maximum of 2.974 g/ 100 in T_3W and 2.980 g/100g in FC by the end of the sixth month. But there was no significant difference in the moisture content of T_3W and FC throughout the storage period.

Titrable acidity

Table 126. Titrable acidity in wheat bran added fermented food mixture and fermented control on storage (g lactic acid/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	3.243 [a]	3.356 [b]	3.247 [c]	3.480 [d]	3.576 [e]	3.643 [f]	3.713 [g]
FC	2.537 [a]	2.607 [b]	2.653 [c]	2.773 [d]	3.027 [e]	3.250 [f]	3.367 [g]
Mean difference	0.706	0.749	0.594	0.707	0.549	0.393	0.346
t value	74.95	79.55	82.02	67.04	73.79	59.00	73.53
Significance	S	S	S	S	S	S	S

T_3W-Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

Titrable acidity was significantly high in T_3W when compared to FC throughout the storage period. Initial titrable acidity of T_3W was 3.243 g lactic acid/100g as against 2.537 g lactic acid/100g in FC. Titrable acidity was significantly increasing in both T_3W and FC with the storage period. By the end of the storage period, a maximum of 3.713 g lactic acid /100 g was observed in T_3W whereas it was 3.367 g lactic acid/100g in FC.

The tirtrable acidity of T_3W (Wheat bran in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 33 for comparison.

Starch

Table 127. Starch content of wheat bran added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	54.550 [a]	53.957 [b]	52.853 [c]	52.160 [d]	50.367 [e]	49.176 [f]	47.863 [g]
FC	54.567 [a]	53.953 [b]	52.850 [c]	52.163 [d]	50.363 [e]	49.183 [f]	47.853 [g]
Mean difference	-0.017	0.004	0.003	-0.003	0.004	-0.007	0.01
T value	-1.147	0.224	0.180	-0.213	0.707	-0.392	0.452
Significance	0.315	0.834	0.866	0.842	0.519	0.715	0.675
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

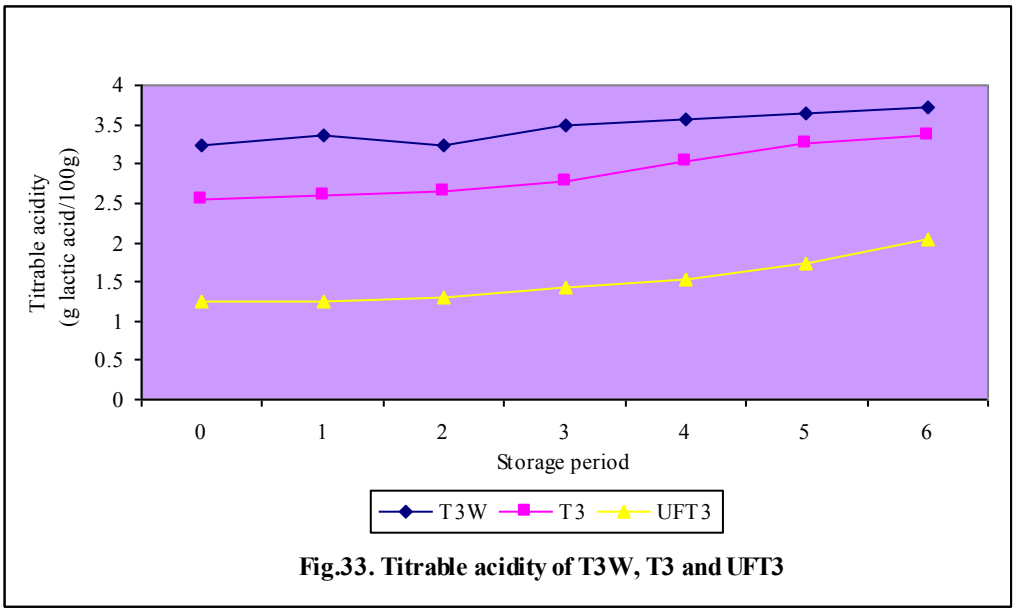


Fig.33. Titrable acidity of T3W, T3 and UFT3

The starch content in T_3W and FC showed a significant reduction with the storage period. Initially the starch content in T_3W was 54.550 g/100g which decreased to 47.863 g /100g by the end of six months. The starch content of T_3W and FC showed no significant difference throughout the storage period

Total soluble solids (TSS)

Table 128. Total soluble solids (TSS) in wheat bran added fermented food mixture and fermented control on storage (°Brix)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	11.651 [a]	11.660 [a]	11.676 [b]	11.706 [b]	11.730 [c]	11.756 [c]	11.800 [d]
FC	11.650 [a]	11.670 [a]	11.680 [b]	11.703 [b]	11.733 [c]	11.751 [c]	11.793 [d]
Mean difference	0.001	-0.01	-0.004	0.003	-0.003	0.005	0.007
T value	0.500	-0.378	-0.267	0.267	-1.00	1.00	0.277
Significance	0.643	0.725	0.802	0.802	0.374	0.374	0.795
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was no significant difference in the TSS content of T_3W upto one month of storage. Initially TSS was 11.651°brix and by the end of the sixth month, TSS was 11.800°brix. There was no significant difference in the TSS content in T_3W and FC throughout the storage period.

Reducing sugar and total sugars

Table 129. Reducing sugar content in wheat bran added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	2.770 [e]	2.776 [e]	2.800 [de]	2.810 [cd]	2.833 [bc]	2.856 [ab]	2.866 [a]
FC	2.766 [ef]	2.773 [e]	2.796 [de]	2.813 [cd]	2.836 [bc]	2.853 [b]	2.866 [a]
Mean difference	0.004	0.003	0.004	-0.003	-0.003	0.003	0.000
t value	0.213	0.243	0.221	-0.500	-0.354	0.267	0.0001
Significance	0.842	0.820	0.836	0.643	0.742	0.802	1.000
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

Reducing sugar in both T_3W and FC showed a significant increase during storage. Initially, reducing sugar content of T_3W was 2.77 g/100g as against 2.766 g/100g in FC. There was no significant difference in the reducing sugar content upto the second month of storage. After two months, the reducing sugar content increased significantly, and by the end of the sixth month, it was 2.866 g/100g in T_3W and FC. There was no significant difference in the reducing sugar content in T_3W and FC throughout the storage period.

Total sugar

Table 130. Total sugar content in wheat bran added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	5.243 ^{d}	5.240 ^{bc}	5.256 ^{a}	5.277 ^{de}	5.286 ^{cd}	5.319 ^{bc}	5.360 ^{b}
FC	5.250 ^{f}	5.243 ^{f}	5.260 ^{e}	5.270 ^{d}	5.300 ^{c}	5.316 ^{b}	5.354 ^{a}
Mean difference	-0.007	-0.003	-0.004	0.007	-0.014	0.003	0.006
t value	-0.500	0.632	-0.632	0.267	-2.00	0.894	1.066
Significance	0.643	0.561	0.561	0.802	0.116	0.422	0.346
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

The total sugar content in T_3W increased to 5.360 g/100 g. There was no significant difference in the reducing sugar content in T_3W and FC throughout the storage period.

Protein

Table 131. Protein content in wheat bran added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	9.816 ^{a}	9.663 ^{b}	9.470 ^{c}	9.076 ^{d}	8.672 ^{e}	8.446 ^{f}	8.257 ^{g}
FC	9.805 ^{a}	9.670 ^{b}	9.450 ^{c}	9.063 ^{d}	8.663 ^{e}	8.433 ^{f}	8.243 ^{g}
Mean difference	0.011	-0.007	0.02	0.013	0.009	0.013	0.014
t value	0.707	-1.000	0.822	1.414	1.061	1.069	1.069
Significance	0.519	0.374	0.457	0.230	0.349	0.345	0.345
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

Initially the protein content in T_3W was 9.816 g/ 100g which decreased significantly with storage and by the end of the sixth month the protein content was 8.257 g/100g as against 8.243 g/100g in FC. There was no significant difference in the protein content of T_3W and FC throughout the storage period

β carotene

Table 132. β carotene content in wheat bran added fermented food mixture and fermented control on storage (µg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	470.920[a]	467.857[b]	463.153[c]	455.230[d]	429.053[e]	402.153[f]	377.160[g]
FC	470.913[a]	467.840[b]	463.156[c]	455.237[d]	429.043[e]	402.170[f]	377.157[g]
Mean difference	0.007	0.017	-0.003	-0.007	0.01	-0.017	0.003
t value	0.500	0.945	-0.302	-0.500	0.557	-0.945	0.164
Significanc	0.643	0.398	0.778	0.643	0.607	0.398	0.877
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

Initially, the β carotene content in T_3W was 470.920 g/100g which decreased significantly on storage and by the end of sixth month, β carotene content in T_3W was 377.160 g/100g. There was no significant difference in the β carotene content of T_3W and FC throughout the storage period.

Calcium

Table 133. Calcium content in wheat bran added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	69.686[a]	69.363[b]	68.420[c]	66.126[c]	63.873[e]	62.213[f]	60.953[g]
FC	69.696[a]	69.373[b]	68.416[c]	66.120[c]	63.870[e]	62.203[f]	60.960[g]
Mean difference	-0.01	-0.01	0.04	0.006	0.003	0.010	-0.007
t value	-0.487	-1.342	0.500	0.500	0.171	1.061	-0.263
Significance	0.652	0.251	0.643	0.643	0.872	0.349	0.806
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

The calcium content in T_3W decreased significantly on storage from 69.686 mg/100g initially, to 60.953 mg/100g by the end of storage. There was no significant difference in the calcium content of T_3W and FC throughout the storage period

Potassium

Table 134. Potassium content of wheat bran added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	396.666[a]	396.256[b]	396.026[c]	394.050[d]	392.156[e]	389.933[f]	377.813[g]
FC	396.670[a]	396.260[b]	396.040[c]	393.046[d]	392.153[e]	389.950[f]	377.823[g]
Mean difference	-0.004	-0.004	-0.014	1.004	0.003	-0.017	-0.01
t value	-0.316	-0.392	-1.000	0.250	0.267	-0.784	-1.060
Significance	0.768	0.715	0.374	0.815	0.802	0.477	0.349
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

The potassium content in T_3W was 396.666 mg/100g initially which decreased significantly on storage and by the end of the sixth month, the potassium content was 377.813 mg/100g. There was no significant difference in the potassium content in T_3W and FC through out the storage period.

Iron

Table 135. Iron content of wheat bran added fermented food mixture and fermented food control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	6.793[a]	6.550[b]	6.366[c]	6.146[d]	6.020[e]	5.866[f]	5.659[g]
FC	6.790[a]	6.553[b]	6.370[c]	6.171[d]	6.060[e]	5.873[f]	5.661[g]
Mean difference	0.003	-0.003	-0.004	-0.025	-0.04	-0.007	-0.002
t value	0.378	-0.447	-0.213	-1.342	-0.378	-0.707	-1.000
Significance	0.725	0.678	0.842	0.251	0.725	0.519	0.374
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant decrease in the iron content in T_3W and FC with storage. The initial iron content in T_3W was 6.793 mg/100g and by the end of the sixth month, the iron content was 5.659 mg/100g. There was no significant difference in the iron content of T_3W and FC throughout the storage period.

Thiamine

Table 136. Thiamine content of wheat bran added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	0.0883^{a}	0.0697^{b}	0.0583^{c}	0.0520^{d}	0.0447^{e}	0.0290^{f}	0.0257^{g}
FC	0.0890^{a}	0.0690^{b}	0.0577^{c}	0.0513^{d}	0.0443^{e}	0.0287^{f}	0.0230^{g}
Mean difference	-0.0007	0.0007	0.0006	0.0007	0.0004	0.0003	0.0027
t value	0.316	0.707	1.414	1.000	0.302	1.000	0.507
Significance	0.768	0.519	0.230	0.374	0.778	0.374	0.639
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

The thiamine content in T3W was 0.0883 mg/100g initially which reduced significantly to 0.0257 mg/100g by the end of the sixth month. There was no significant difference in the thiamine content of T3W and FC throughout the storage period.

Riboflavin

Table 137. Riboflavin content of wheat bran added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	0.623^{a}	0.423^{b}	0.350^{c}	0.333^{d}	0.283^{e}	0.246^{f}	0.156^{g}
FC	0.610^{a}	0.416^{b}	0.343^{c}	0.326^{d}	0.280^{e}	0.240^{f}	0.150^{g}
Mean difference	0.013	0.013	0.007	0.007	0.003	0.006	0.006
t value	0.707	0.707	1.000	0.707	0.378	0.632	0.500
Significance	0.519	0.519	0.374	0.519	0.725	0.561	0.643
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

The riboflavin content in T_3W was 0.623 mg/100g initially which reduced to 0.156 mg/100g by six months of storage. There was no significant difference in the thiamine content of T_3W compared to FC throughout the storage period.

In vitro starch digestibility (IVSD)

Table 138. IVSD of wheat bran added fermented food mixture and fermented control on storage (percentage)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	80.630 ^{d}	80.633 ^{d}	81.300^{c}	81.466 ^{c}	82.433^{b}	82.623^{b}	83.366^{a}
FC	80.633 ^{e}	80.700 ^{e}	81.201^{d}	81.400 ^{d}	82.333^{c}	82.630^{b}	83.233^{a}
Mean difference	-0.003	-0.067	0.099	0.066	0.1	-0.007	0.133
T value	-0.229	-1.732	0.707	1.604	0.626	-0.267	0.784
Significance	0.830	0.158	0.519	0.184	0.166	0.802	0.477
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant increase in the IVSD of T_3W and FC on storage. The IVSD was 80.630 per cent initially in T_3W, which increased significantly on storage and by the end of six months, the IVSD in T_3W was 83.366 per cent. There was no significant difference in the IVSD of T_3W and FC throughout the storage period**.**

In vitro protein digestibility (IVPD)

Table 139. IVPD of wheat bran added fermented food mixture and fermented control on storage (percentage)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3W	85.566 ^{f}	85.573 ^{f}	85.850^{e}	86.213 ^{d}	86.930^{c}	87.123^{b}	87.577^{a}
FC	85.556 ^{f}	85.580 ^{f}	85.850^{e}	86.206 ^{d}	86.937^{c}	87.133^{b}	87.586^{a}
Mean difference	0.01	-0.007	0.0001	0.007	-0.007	-0.01	-0.011
t value	0.671	-0.311	0.0001	0.447	-0.316	0.671	0.469
Significance	0.539	0.766	1.000	0.678	0.768	0.539	0.664
	NS	NS	NS	NS	NS	NS	NS

T_3W– Wheat bran in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant increase in IVPD of T_3W and FC during storage. The IVPD in T_3W was 85.566 per cent initially which increased significantly on storage and by the end of six months, the IVPD was 87.577 per cent. There was no significant difference in the IVPD of T_3W and FC throughout the storage period.

4.9.2.1.4. Chemical constituents in fermented food with skimmed milk powder on storage

Moisture

Table 140. Moisture content of skimmed milk powder added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	1.836 [a]	1.837 [a]	1.903 [b]	2.138 [c]	2.242 [d]	2.543 [e]	2.973 [f]
FC	1.837 [a]	1.843 [a]	1.903 [b]	2.140 [c]	2.243 [d]	2.547 [e]	2.980 [f]
Mean difference	-0.001	-0.006	0.0001	-0.002	-0.001	-0.004	-0.009
T value	-0.186	-2.289	0.0001	-0.288	-2.99	-1.194	-1.109
Significance	0.862	0.084	1.000	0.788	0.780	0.298	0.329
	NS	NS	NS	NS	NS	NS	NS

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

The moisture content showed no significant difference upto one month of storage but increased significantly throughout the storage period in both T_3SK and FC. After six months of storage, moisture content of T_3SK was 2.973 g/ 100. But there was no significant difference in the moisture content of T_3SK and FC throughout the storage period.

Titrable acidity

Table 228. Titrable acidity in skimmed milk powder added fermented food mixture and fermented control on storage (g lactic acid/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	3.543 [a]	3.610 [b]	3.650 [c]	3.780 [d]	3.900 [e]	4.240 [f]	4.370[g]
FC	2.537 [a]	2.607 [b]	2.653 [c]	2.773 [d]	3.027 [e]	3.250 [f]	3.367[g]
Mean difference	1.006	1.003	0.997	1.007	0.873	0.99	1.003
t value	-69.75	-115.38	-92.99	-58.04	-60.01	-128.35	-76.27
Significance	S	S	S	S	S	S	S

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

Titrable acidity was significantly increasing with the storage period in T_3SK Initially titrable acidity was 3.543 g lactic acid /100 g and by the end of the storage period it reached to a maximum of 4.370 g lactic acid /100 g as against 3.367 g/100g in FC. There was a significant increase in the titrable acidity of T3SK compared to FC throughout the storage period.

The titrable acidity of T_3SK (Skimmed milk powder in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 34 for comparison.

Starch

Table 142. Starch content in skimmed milk powder added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	54.556 [a]	53.922 [b]	52.864[c]	52.163 [d]	50.364[e]	49.192[f]	47.869[g]
FC	54.567 [a]	53.953 [b]	52.850[c]	52.163 [d]	50.363[e]	49.183[f]	47.853[g]
Mean difference	-0.011	-0.031	0.014	0.0001	0.001	0.009	0.016
t value	-1.246	-0.605	1.241	0.028	0.099	0.619	1.055
Significance	0.281	0.578	0.282	0.979	0.780	0.569	0.351
	NS	NS	NS	NS	NS	NS	NS

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

The starch content in T_3SK and FC showed a significant reduction with the storage period. Initially the starch content in T_3SK was 54.556 g/100g which decreased to 47.869 g /100g by the end of the six months. The starch content of T_3SK and FC showed no significant difference throughout the storage period

Total soluble solids

Table 143.Total soluble solids (TSS) in skimmed milk powder added fermented food mixture and fermented control on storage (°Brix)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	12.286 [a]	12.227 [a]	12.367 [b]	12.376 [b]	12.403 [c]	12.467 [c]	12.547 [d]
FC	11.650 [a]	11.670 [a]	11.680 [b]	11.703 [b]	11.733 [c]	11.751 [c]	11.793 [d]
Mean difference	0.636	0.557	0.687	0.673	0.67	0.716	0.754
t value	84.523	17.945	73.539	71.064	141.421	0.212	70.835
Significance	S	S	S	S	S	S	S

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant increase in the TSS of T_3SK and FC during storage. Initially TSS of T_3SK was 12.286°brix which increased significantly to 12.547°brix after six months of storage as against 11.793°brix in FC. T_3SK showed significantly higher value for TSS when compared to FC throughout the storage period.

The TSS of T_3SK (Skimmed milk powder in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 35 for comparison.

Reducing sugars and total sugars

Table 144. Reducing sugar content in skimmed milk powder added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	4.017[e]	4.037 [de]	4.087 [d]	4.113 [cd]	4.137 [c]	4.213 [b]	4.483 [a]
FC	2.766 [ef]	2.773 [e]	2.796 [de]	2.813 [cd]	2.836 [bc]	2.853 [b]	2.866 [a]
Mean difference	1.251	1.264	1.291	1.300	1.301	1.360	1.617
t value	100.223	267.993	173.072	275.772	275.772	288.500	129.622
Significance	S	S	S	S	S	S	S

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

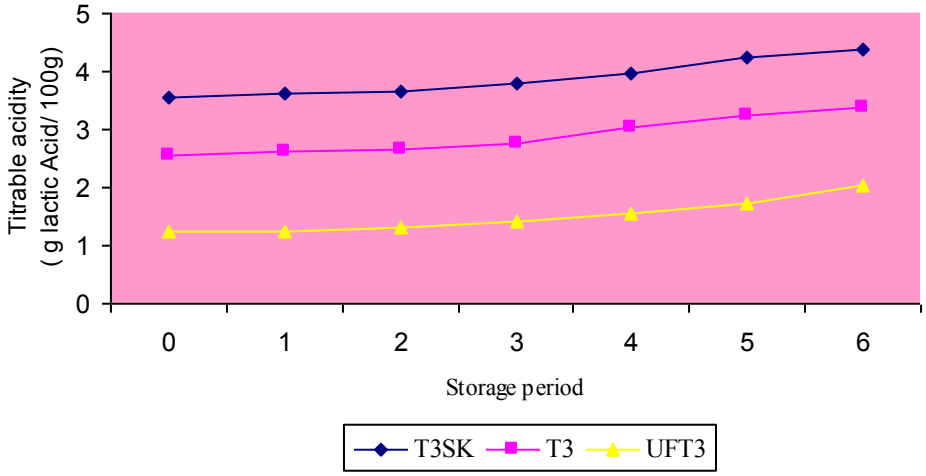


Fig. 34.Titrable acidity of T3SK, T3 and UFT3

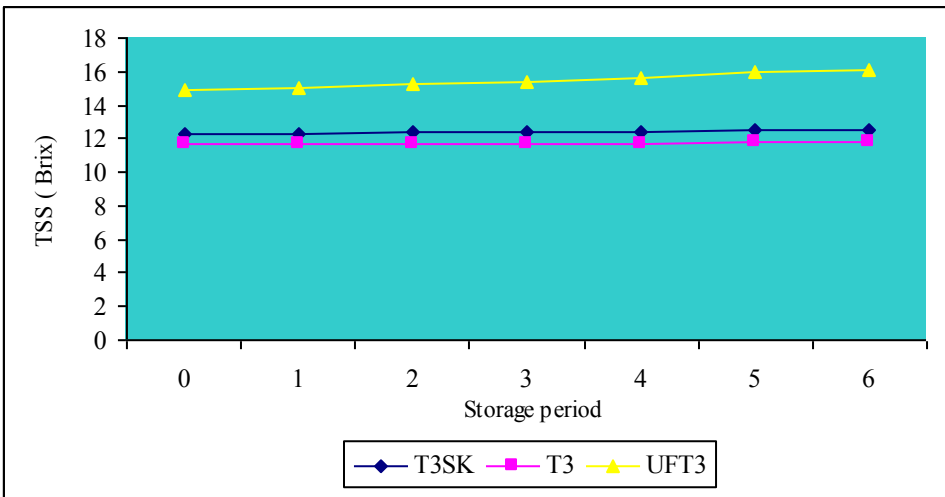


Fig.35.TSS of T3SK, T3 and UFT3

In both T_3SK and FC, reducing sugar increased significantly with storage. Initially, reducing sugar content of T_3SK was 4.017 g/100g, which increased significantly and by the end of the sixth month, it was 4.483 g/100g as against 2.866 g/100g in FC. There was a significant increase in the reducing sugar content in T_3SK compared to FC throughout the storage period.

The reducing sugar of T_3SK (Skimmed milk powder in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 36 for comparison.

.

Total sugar

Table 145. Total sugar content in skimmed milk powder added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	6.560 ^{f}	6.817 ^{e}	6.837 ^{d}	6.827 ^{d}	6.846 ^{c}	6.876 ^{b}	6.923 ^{a}
FC	5.250 ^{f}	5.243 ^{f}	5.260 ^{e}	5.270 ^{d}	5.300 ^{c}	5.316 ^{b}	5.354 ^{a}
Mean difference	1.310	1.574	1.577	1.557	1.546	1.560	1.569
t value	160.442	321.026	113.714	125.346	232.000	112.779	105.095
Significance	S	S	S	S	S	S	S

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

The total sugar content in T_3SK and FC increased significantly with storage. The initial total sugar in T_3SK was 6.560 g/100g and in FC it was 5.250 g/100g. By the end of sixth month the reducing sugar content in T_3SK was significantly increased to 6.923 g /100g as against 5.354 g/100g in FC. There was a significant increase in the reducing sugar content in T_3SK compared to FC throughout the storage period.

The total sugar of T_3SK (Skimmed milk powder in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 37 for comparison.

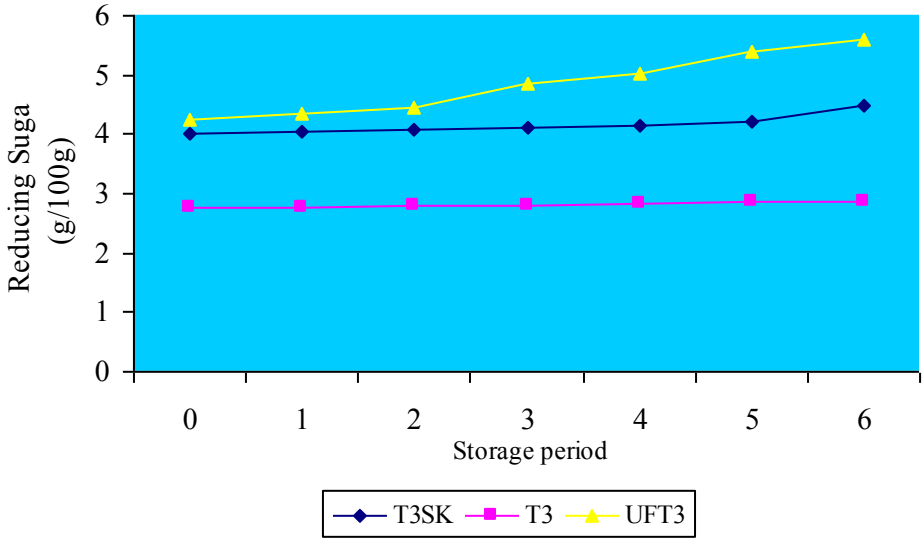


Fig. 36. Reducing Sugar in T3SK, T3 and UFT3

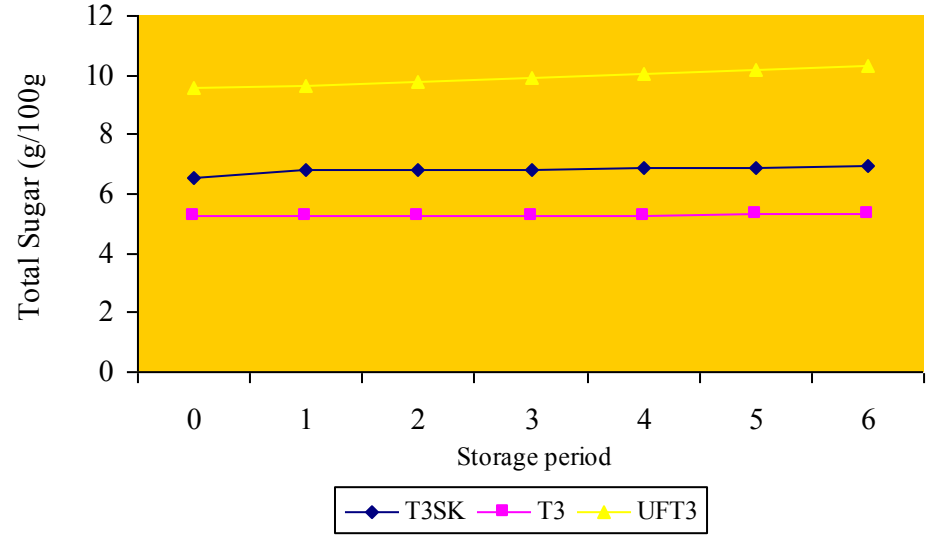


Fig.37. Total sugar in T3SK, T3 and UFT3

Protein

Table 146. Protein content in skimmed milk powder added fermented food mixture and fermented control on storage (g/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	9.987 [a]	9.877 [b]	9.678 [c]	9.223 [d]	8.981 [e]	8.776 [f]	8.467 [g]
FC	9.805 [a]	9.670 [b]	9.450 [c]	9.063 [d]	8.663 [e]	8.433 [f]	8.243 [g]
Mean difference	0.182	0.207	0.228	0.160	0.318	0.343	0.224
t value	22.841	34.990	60.820	23.970	94.927	38.865	25.306
Significance	S	S	S	S	S	S	S

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant reduction in protein content of T_3SK and FC during storage. Initially, the protein content in T_3SK was 9.987 g/100g which decreased significantly with storage and by the end of the sixth month, the protein content was 8.467 g/100g as against 8.243 g/100g in FC. There was a significant increase in the protein content of T_3SK compared to FC throughout the storage period

The protein of T_3SK (Skimmed milk powder in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 38 for comparison.

ß carotene

Table 147. β carotene content in skimmed milk powder added fermented food mixture and fermented control on storage (µg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	470.953[a]	467.863[b]	463.173[c]	455.253[d]	429.063[e]	402.180[f]	377.183[g]
FC	470.913[a]	467.840[b]	463.156[c]	455.237[d]	429.043[e]	402.170[f]	377.157[g]
Mean difference	0.04	0.023	0.017	0.016	0.02	0.01	0.026
t value	2.211	2.214	2.236	2.236	1.455	0.866	1.940
Significance	0.093	0.091	0.089	0.089	0.219	0.435	0.124
	NS	NS	NS	NS	NS	NS	NS

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant reduction in the β carotene content of both T_3SK and FC during storage. Initially, the β carotene content in T_3SK was 470.953 g/100g which reduced significantly on storage and by the end of the sixth month, β carotene content in T_3SK was 377.183 g/100g as against 377.157 µg/100g in FC. There was no significant difference in the β carotene content of T_3SK and FC throughout the storage period.

Calcium

Table 148.Calcium content in skimmed milk powder added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	71.217[a]	71.137[b]	70.183[c]	68.687[c]	65.973[e]	64.717[f]	62.807[g]
FC	69.696[a]	69.373[b]	68.416[c]	66.120[c]	63.870[e]	62.203[f]	60.960[g]
Mean difference	1.574	1.764	1.767	2.567	2.103	2.514	1.847
t value	161.22	374.059	374.767	385.000	134.530	33.159	118.113
Significance	S	S	S	S	S	S	S

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant reduction in calcium content of both T_3SK and FC on storage. The calcium content in T_3SK decreased significantly on storage from 71.217 mg/100g to 62.807 mg/100g by the end of storage. There was a significant increase in the calcium content of T_3SK compared to FC throughout the storage period.

The calcium of T_3SK (Skimmed milk powder in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 39 for comparison.

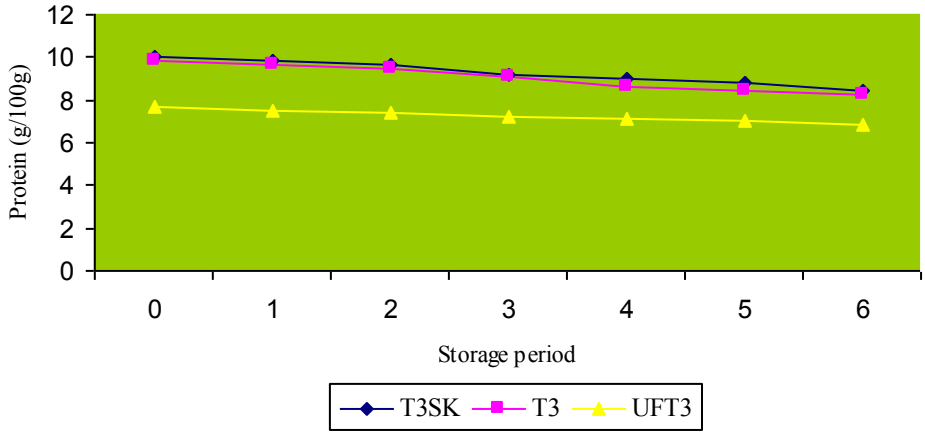


Fig. 38. Protein content in T3SK, T3 and UFT3

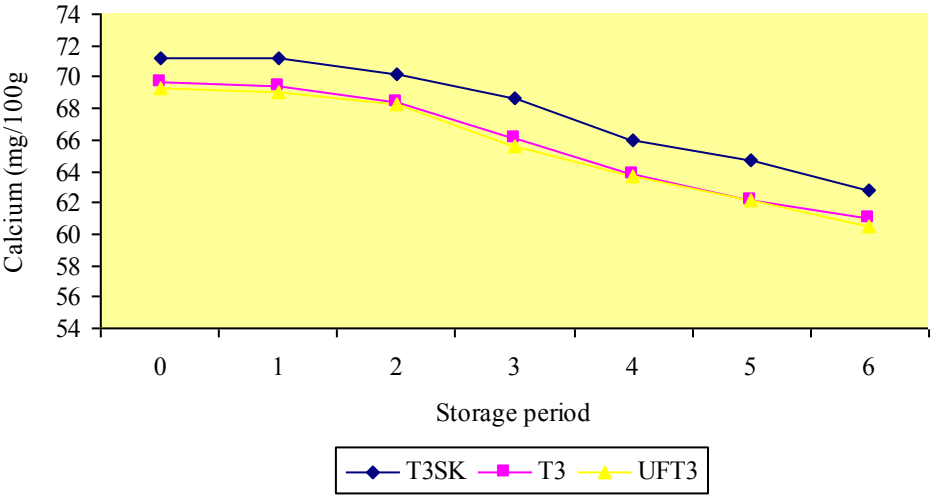


Fig. 39. Calcium content of T3SK, T3 and UFT3

Potassium

Table 149. Potassium content of skimmed milk powder added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	401.433[a]	401.033[b]	400.917[c]	400.027[d]	398.117[e]	397.230[f]	385.887[g]
FC	396.670[a]	396.260[b]	396.040[c]	393.046[d]	392.153[e]	389.950[f]	377.823[g]
Mean difference	4.763	4.773	4.877	6.981	5.964	7.280	8.064
t value	35.755	505.935	935.204	634.275	632.507	82.852	855.246
Significance	S	S	S	S	S	S	S

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

Potassium content in both T_3SK and FC reduced significantly with storage. The potassium content in T_3SK was 401.433 mg/100g initially as against 396.670 mg/100g in FC. This decreased significantly on storage and by the end of the sixth month, potassium content was 385.887 mg/100g in T_3SK and 377.823 mg/100g in FC. There was a significant increase in the potassium content of T_3SK compared to FC throughout the storage period.

The potassium of T_3SK (Skimmed milk powder in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 40 for comparison.

Iron

Table 150. Iron content of skimmed milk powder added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	7.147[a]	7.103[b]	7.067[c]	7.023[d]	7.003[e]	6.157[f]	6.137[g]
FC	6.790[a]	6.553[b]	6.370[c]	6.171[d]	6.060[e]	5.873[f]	5.661[g]
Mean difference	0.357	0.553	0.697	0.853	0.943	0.287	0.477
t value	55.000	73.343	66.092	161.276	132.375	38.013	97.581
Significance	S	S	S	S	S	S	S

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant decrease in the iron content of T_3SK and FC with storage. The initial iron content in T_3SK was 7.147 mg/100g and by the end of the sixth month, the iron content was 6.137 mg/100g. Iron content of T_3SK was significantly higher than FC throughout the storage period.

The iron content of T_3SK (Skimmed milk powder in T_3), T_3 and UFT_3 (unfermented T3) is depicted in Fig 41 for comparison.

Thiamine

Table 151. Thiamine content of skimmed milk powder added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	0.1017^{a}	0.0977^{b}	0.0877^{c}	0.0803^{d}	0.0677^{e}	0.0607^{f}	0.0557^{g}
FC	0.0890^{a}	0.0690^{b}	0.0577^{c}	0.0513^{d}	0.0443^{e}	0.0287^{f}	0.0230^{g}
Mean difference	0.0127	0.0287	0.0297	0.0293	0.0237	0.0317	0.0327
t value	20.500	60.104	63.640	61.518	31.305	67.882	9.652
Significance	S	S	S	S	S	S	S

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant reduction in the thiamine content of T_3SK and FC on storage. The thiamine content in T_3SK was 0.1017 mg/100g initially, which reduced significantly to 0.0557 mg/100g by the end of the sixth month. Thiamine content of T_3SK was significantly high when compared to FC throughout the storage period.

The thiamine content of T_3SK (Skimmed milk powder in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 42 for comparison.

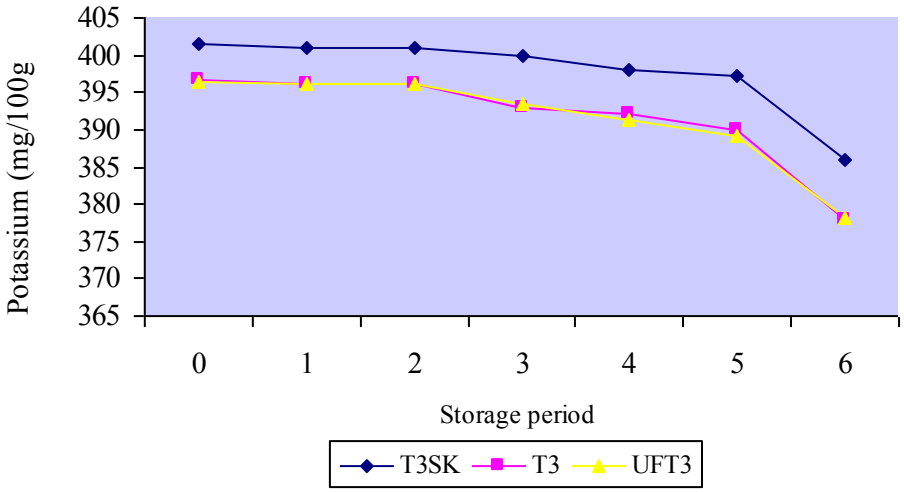


Fig. 40. Potassium content of T3SK, T3 and UFT3

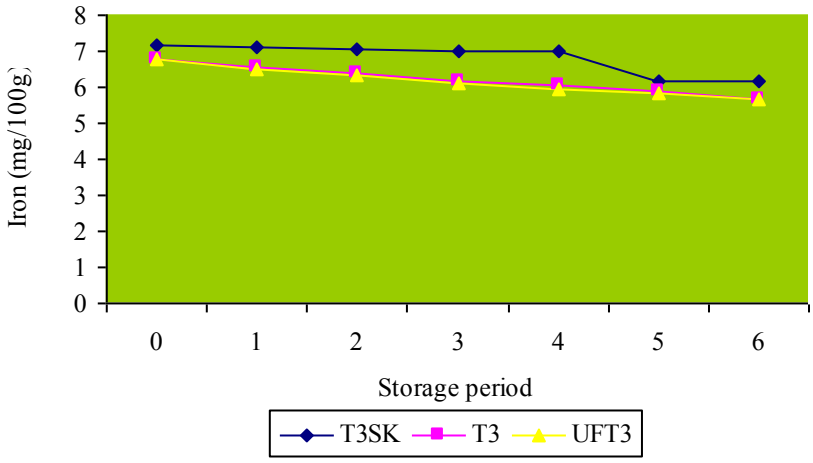


Fig. 41. Iron content of T3SK, T3 and UFT3

Riboflavin

Table 152. Riboflavin content of skimmed milk powder added fermented food mixture and fermented control on storage (mg/100g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	0.907^{a}	0.823^{b}	0.787^{c}	0.703^{d}	0.603^{e}	0.457^{f}	0.427^{g}
FC	0.610^{a}	0.416^{b}	0.343^{c}	0.326^{d}	0.280^{e}	0.240^{f}	0.150^{g}
Mean difference	0.297	0.413	0.447	0.373	0.323	0.217	0.277
t value	61.518	86.267	94.945	39.952	48.500	32.500	41.500
Significance	S	S	S	S	S	S	S

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

The riboflavin content in T_3SK was 0.907 mg/100g initially, which reduced significantly to 0.427 mg/100g by six months of storage. There was a significant increase in the riboflavin content of T_3SK, which was significantly high when compared to FC throughout the storage period.

The riboflavin content of T_3SK (Skimmed milk powder in T_3), T_3 and UFT_3 (unfermented T_3) is depicted in Fig 43 for comparison.

***In vitro* starch digestibility (IVSD)**

Table 153.IVSD of skimmed milk powder added fermented food mixture and fermented control on storage (percentage)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	80.566^{e}	80.720^{e}	81.133^{d}	81.447^{d}	82.233^{c}	82.533^{b}	83.133^{a}
FC	80.633^{e}	80.700^{e}	81.201^{d}	81.400^{d}	82.333^{c}	82.630^{b}	83.233^{a}
Mean difference	-0.064	0.02	-0.067	0.047	-0.097	-0.097	-0.097
t value	-0.555	0.755	-0.632	0.561	-1.342	-1.414	-1.061
Significance	0.609	0.482	0.561	0.601	0.251	0.230	0.349
	NS	NS	NS	NS	NS	NS	NS

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

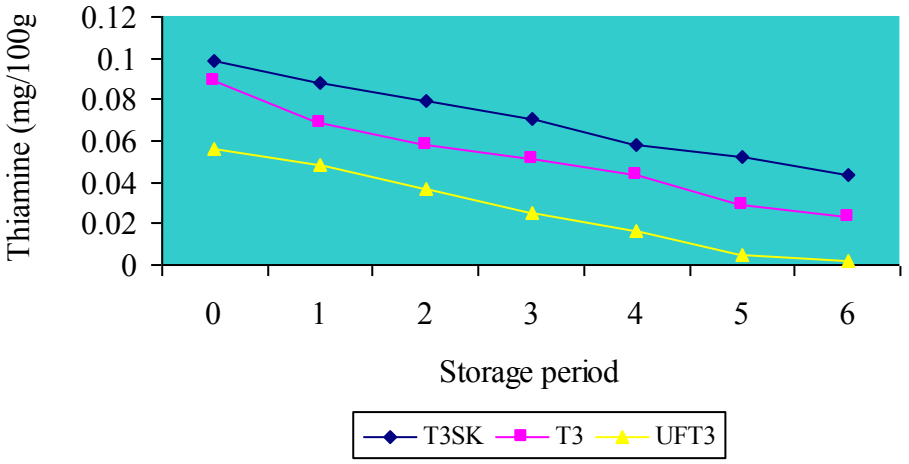


Fig. 42. Thiamine content of T3SK, T3 and UFT3

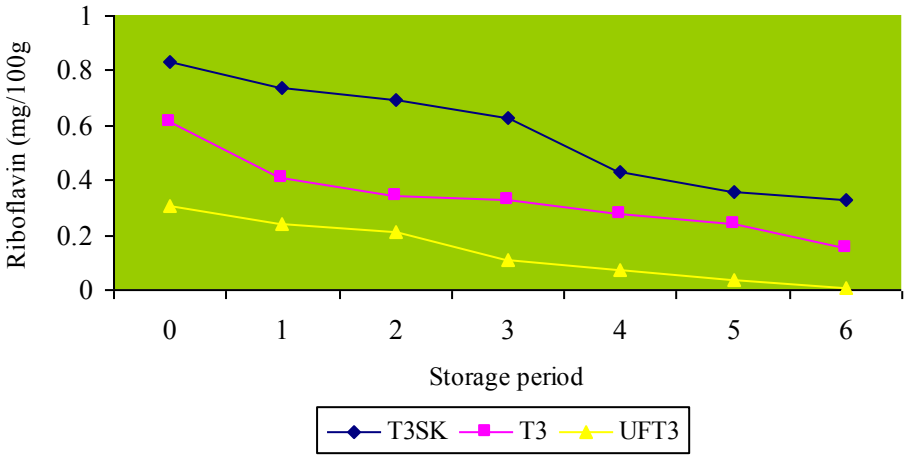


Fig. 43. Riboflavin content of T3SK, T3 and UFT3

There was a significant increase in IVSD of T_3SK and FC with storage. The IVSD was 80.566 per cent initially in T_3SK which increased significantly on storage and by the end six months, the IVSD in T_3SK was 83.133 per cent. There was no significant difference in the IVSD of T_3SK and FC throughout the storage period**.**

***In vitro* protein digestibility (IVPD)**

Table 154. IVPD of skimmed milk powder added fermented food mixture and fermented control on storage (percentage)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
T_3SK	85.580 ^{f}	85.620 ^{f}	85.523 ^{e}	86.193 ^{d}	86.946 ^{c}	87.150 ^{b}	87.606 ^{a}
FC	85.556 ^{f}	85.580 ^{f}	85.850 ^{e}	86.206 ^{d}	86.937 ^{c}	87.133 ^{b}	87.586 ^{a}
Mean difference	0.02	0.04	-0.327	-0.017	0.006	0.02	0.016
T value	1.750	1.250	-0.968	-1.414	0.469	1.250	1.114
Significance	0.155	0.279	0.388	0.230	0.664	0.279	0.238
	NS	NS	NS	NS	NS	NS	NS

T_3SK– Skimmed milk powder in T_3
Values having different super script differ significantly at 5% level
DMRT row wise comparison

There was a significant increase in the IVPD of T_3SK and FC with storage. The IVPD in T_3SK was 85.580 per cent initially, which increased significantly on storage and by the end of six months, the IVPD was 87.606 per cent. There was no significant difference in the IVPD of T_3SK and FC throughout the storage period.

4.9.2.2. Total microbial count and viability of *L. acidophilus* in the modified food mixtures on storage.

4.9.2.2.1. Total microbial population in the modified food mixtures on storage.

Modified food mixtures such as T_3S (sucrose in T_3), T_3SB (sorbitol in T_3), T3W (wheat bran in T_3) and T_3SK (skimmed milk powder in T_3) and their fermented control (T_3) were enumerated for total bacteria, fungi and yeast during each month and the results are presented in Table 155 and 156.

4.9.2.2.1.1. Total bacterial count in modified food mixtures on storage

Table 155. Total Bacterial count in the modified food mixtures ($\times 10^7$ cfu/ g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
FC	283[a] (9.45)	273[b] (9.43)	257[c] (9.41)	223[d] (9.34)	207[e] (9.31)	189[f] (9.28)	135[g] (9.13)
T_3S	352[a] (9.55)	346[b] (9.54)	323[c] (9.51)	290[d] (9.46)	269[e] (9.43)	247[f] (9.39)	211[g] (9.32)
T_3SB	286[a] (9.46)	273[b] (9.44)	255[c] (9.40)	223[d] (9.34)	208[e] (9.13)	192[f] (9.28)	137[g] (9.14)
T3W	336[a] (9.53)	324[b] (9.51)	303[c] (9.48)	282[d] (9.45)	253[e] (9.40)	231[f] (9.36)	193[g] (9.28)
T_3SK	350[a] (9.54)	338[b] (9.53)	316[c] (9.50)	289[d] (9.46)	264[e] (9.42)	242[f] (9.38)	198[g] (9.29)

(Figures in parenthesis are log cfu/g)
T_3S- sucrose in T_3, T_3SB- sorbitol in T_3, T3W- wheat bran in T_3, T_3SK- skimmed milk powder in T_3

The bacterial count decreased significantly on storage in all the treatments. The initial bacterial counts in all the modified treatments were more than FC. Among the modified food mixtures, initially maximum bacterial count was in T_3S (352 $\times 10^7$ cfu/g) and minimum in T_3SB (286 $\times 10^7$ cfu/g). The bacterial count decreased on storage and by the end of sixth months, maximum count was in T_3S (211 $\times 10^7$ cfu/g) and minimum in T_3SB (137 $\times 10^7$ cfu/g).

4.9.2.2.1.2. Total fungal count in modified food mixtures on storage.

Table 156. Fungal count in the modified food mixtures ($\times 10^3$ cfu/ g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
FC	Nil	1.0	1.0	1.3	1.5	2.0	2.1
T_3S	Nil	1.0	1.0	1.0	1.0	1.0	1.1
T_3SB	Nil	1.8	1.8	1.8	1.8	1.8	1.8
T3W	Nil	2.0	2.0	2.0	2.0	2.0	2.0
T_3SK	Nil	1.8	2.0	2.0	2.0	2.0	2.0

T_3S- sucrose in T_3, T_3SB- sorbitol in T_3, T3W- wheat bran in T_3, T_3SK- skimmed milk powder in T_3

Initially, in all the treatments there was no fungal growth. After one month fungal growth in FC and T_3S was 1.0 x 10^3 cfu/g, whereas maximum fungal growth was in T_3W (2.0 x 10^3 cfu/g). The fungal growth increased on storage and by the end of the sixth month, maximum fungal growth was in FC (2.1 x10^3 cfu/g) followed by T_3W and T_3SK (2.0×10^3 cfu/g) and minimum in T_3S (1.1 ×10^3 cfu/g).

4.9.2.2.1.3. Yeast count in modified food mixtures on storage.

There was no traces of yeast in any of the modified food mixtures on storage.

4.9.2.2.1.4. Insect infestation of the modified food mixtures on storage.

No insect infestation was observed in any of the modified food mixture on storage.

4.9.2.2.2. Viable count of *L. acidophilus* in the modified food mixtures on storage.

Table 157.Viable count of *L. acidophilus* in modified food mixtures on storage (×10^7 cfu / g)

Treatment	Storage period in months						
	Initial	1	2	3	4	5	6
FC	282[a] (9.45)	271[b] (9.43)	255[c] (9.40)	221[d] (9.33)	205[e] (9.31)	187[f] (9.27)	133[g] (9.12)
T_3S	351[a] (9.54)	344[b] (9.53)	321[c] (9.50)	287[d] (9.45)	267[e] (9.43)	245[f] (9.38)	208[g] (9.32)
T_3SB	284[a] (9.45)	271[b] (9.43)	253[c] (9.39)	220[d] (9.33)	205[e] (9.31)	189[f] (9.27)	134[g] (9.14)
T3W	333[a] (9.52)	321[b] (9.50)	300[c] (9.47)	279[d] (9.44)	250[e] (9.39)	227[f] (9.35)	189[g] (9.28)
T_3SK	347[a] (9.54)	335[b] (9.52)	313[c] (9.49)	286[d] (9.45)	260[e] (9.41)	238[f] (9.37)	194[g] (9.30)

(Figures in parenthesis are log cfu/g)
T_3S- sucrose in T_3, T_3SB- sorbitol in T_3, T3W- wheat bran in T_3, T_3SK- skimmed milk powder in T_3,Values having different super script differ significantly at 5% level
DMRT row wise comparison

Initially, maximum viable count of *L.acidophilus* was observed in T_3S (351 x10^7cfu/g) followed by T_3SK (351 x 10^7cfu/g). During storage there was a considerable

reduction in the viable count of *L. acidophilus*. After six months of storage, maximum viable count was observed in T_3S (208 x 10^7 cfu/g) and T_3SK (194 x cfu/g x 10^7). However T3W and T_3SB also showed higher viable counts than FC after six months of storage. (Fig 44)

4.9.2.3. Organoleptic qualities of the modified food mixtures on storage.

The results of the organoleptic qualities of the modified food mixtures are presented in Table 158 to 161. (Fig 45)

4.9.2.3.1. Organoleptic qualities of sucrose added fermented food mixture on storage

The mean score for appearance of FC and T_3S found to be 8.33 and 8.36 initially (Table 158) which decreased on storage and after six months, the score was 8.10 and 8.11 respectively. With respect to colour, FC and T_3S had a mean score 8.56 and 8.57 respectively which on storage decreased to 8.36 and 8.35 after six months. The flavour of FC and T_3S was 7.10 and 7.50 initially and both in FC and T_3S, there was a reduction in the flavour. By the end of the storage period, the flavour content in FC and T_3S was 6.61 and 7.15 respectively. Initially, the mean score for texture of FC and T_3S was 8.31 which decreased on storage to 8.00 and 8.04 respectively. With regard to taste, T_3S had a high score of 8.45 initially which decreased on storage to 7.78. The taste for FC was less (7.53) compared to T_3S, which decreased on storage and after six months the mean score for taste was 6.87 in FC. Considering all the criteria, Overall acceptability of T_3S ranged from an initial mean score of 8.24 to a mean score of 7.886 after six months. In FC this was 7.966 initially and after six months 7.588.

4.9.2.3.2. Organoleptic qualities of sorbitol added fermented food mixture on storage

Initially, the mean score for appearance of FC and T_3SB was 8.33, which decreased on storage and after six month the score was 8.10 in both (Table 159). With respect to colour, FC and T_3SB had a mean score 8.56 and 8.55 respectively which on

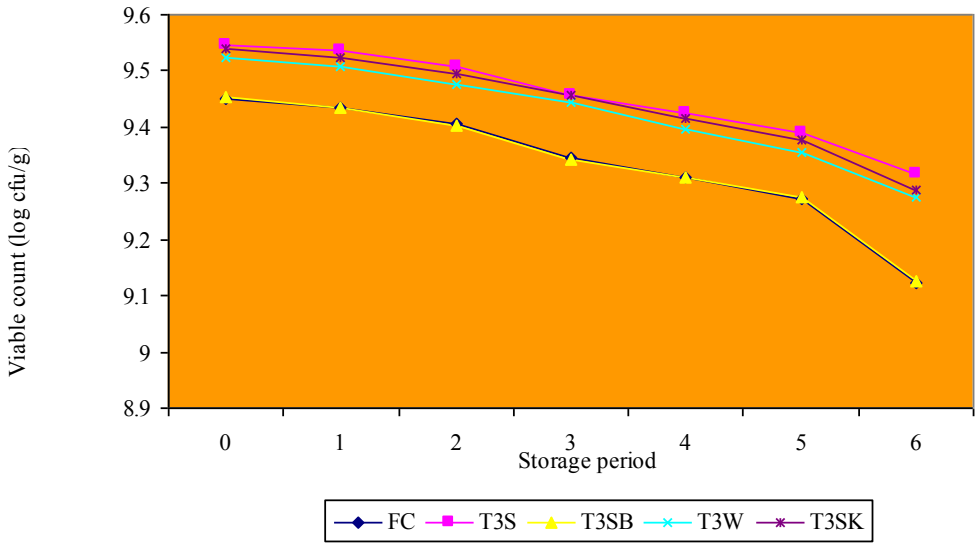


Fig.44. Viable count of *L.acidophilus* in modified food mixtures on storage

Table 158. Mean score for organoleptic qualities of sucrose added fermented food mixture and fermented control on storage.

Quality attributes	Storage period in months													
	Initial		1		2		3		4		5		6	
	FC	T3S	FC	T3S	FC	T3S	FC	T3S	FC	T3S	FC	T3S	FC	T3S
Appearance	8.33	8.36	8.33	8.36	8.31	8.33	8.27	8.30	8.20	8.25	8.15	8.17	8.10	8.11
Colour	8.56	8.57	8.55	8.56	8.53	8.53	8.48	8.48	8.44	8.42	8.40	8.38	8.36	8.35
Flavour	7.10	7.50	7.10	7.50	7.03	7.45	6.86	7.36	6.80	7.30	6.70	7.23	6.61	7.15
Texture	8.31	8.31	8.31	8.32	8.29	8.31	8.27	8.28	8.16	8.18	8.06	8.09	8.00	8.04
Taste	7.53	8.45	7.53	8.43	7.50	8.40	7.43	8.30	7.13	8.03	6.99	7.85	6.87	7.78
OAA	7.966	8.24	7.964	8.234	7.932	8.204	7.862	8.144	7.746	8.036	7.66	7.944	7.588	7.886
Total Score	47.796	49.44	47.784	49.404	47.592	49.224	47.172	48.864	46.476	48.216	45.96	47.664	45.528	47.316

FC- Fermented control, T3S – Sucrose in T3

Table 159. Mean score for organoleptic qualities of sorbitol added fermented food mixture and fermented control on storage.

Quality attributes	Storage period in months													
	Initial		1		2		3		4		5		6	
	FC	T3SB	FC	T3SB	FC	T3SB	FC	T3SB	FC	T3SB	FC	T3SB	FC	T3SB
Appearance	8.33	8.33	8.33	8.32	8.31	8.30	8.27	8.27	8.20	8.19	8.15	8.14	8.10	8.10
Colour	8.56	8.55	8.55	8.55	8.53	8.52	8.48	8.49	8.44	8.45	8.40	8.40	8.36	8.37
Flavour	7.10	7.20	7.10	7.20	7.03	7.15	6.86	6.99	6.80	6.93	6.70	6.81	6.61	6.73
Texture	8.31	8.32	8.31	8.31	8.29	8.30	8.27	8.29	8.16	8.18	8.06	8.08	8.00	8.03
Taste	7.53	8.23	7.53	8.22	7.50	8.18	7.43	8.10	7.13	7.86	6.99	7.73	6.87	7.67
OAA	7.966	8.126	7.964	8.12	7.932	8.09	7.862	8.028	7.746	7.922	7.66	7.832	7.588	7.78
Total Score	47.796	48.756	47.784	48.72	47.592	48.54	47.172	48.168	46.476	47.532	45.96	46.992	45.528	46.68

FC- Fermented control, T3SB – Sorbitol in T3

storage decreased to 8.36 and 8.37 after six both. The flavour of FC and T_3SB was 7.10 and 7.20 initially and in both FC and T_3SB, there was a reduction in the flavour. By the end of the storage period, the mean score for flavour in FC and T_3S was 6.61 and 6.73 respectively. The texture of FC and T_3SB was 8.31 and 8.32 initially and decreased on storage to 8.00 and 8.03 respectively. With regard to taste T_3SB had a high score of 8.23 initially as against 7.53 in FC which decreased on storage to 7.67. The taste for FC was less (7.53) compared to T_3SB, which decreased on storage and after six months the mean score for taste of FC was 6.87. Overall acceptability of T_3SB ranged from an initial score of 8.126 to a mean score of 7.78 whereas in FC this was 7.966 and 7.588 respectively

4.9.2.3.3. Organoleptic qualities of wheat bran added fermented food mixture on storage

As shown in Table 160, the mean score for appearance of FC and T3W was 8.33 and 8.32 initially which decreased on storage and after six months, the score was 8.10. With respect to colour, FC and T3W had a mean score 8.54, which on storage decreased to 8.36 and 8.37 after six months. The flavour of FC and T3W was 7.10 and 7.33 initially and both in FC and T3W, there was a reduction in the flavour. By the end of the storage period, the mean score for flavour in FC and T3W was 6.61 and 6.73 respectively. The texture of FC and T3W was 8.31 initially and decreased on storage to 8.00 and 8.01 respectively. With regard to taste T3W had a high score of 8.20 initially as against 7.53 in FC. This was decreased on storage to 7.67 and 6.87 respectively. The mean score for taste of FC was less (7.53) compared to T3W which decreased on storage and after six months the mean score was 6.87 in FC. Overall acceptability was also higher in T3W which ranged from an initial mean score of 8.144 to 7.774 after six months storage, whereas this was 7.962 and 7.584 in FC

Table 160. Mean score for organoleptic qualities of wheat bran added fermented food mixture and fermented control on storage.

Quality attributes	Storage period in months													
	Initial		1		2		3		4		5		6	
	FC	T3W	FC	T3W	FC	T3W	FC	T3W	FC	T3W	FC	T3W	FC	T3W
Appearance	8.33	8.32	8.33	8.32	8.31	8.30	8.27	8.27	8.20	8.19	8.15	8.14	8.10	8.10
Colour	8.54	8.54	8.54	8.54	8.53	8.51	8.48	8.46	8.44	8.44	8.40	8.39	8.36	8.37
Flavour	7.10	7.33	7.10	7.33	7.03	7.16	6.86	6.98	6.80	6.93	6.70	6.81	6.61	6.73
Texture	8.31	8.31	8.31	8.31	8.29	8.30	8.27	8.27	8.16	8.16	8.06	8.06	8.00	8.01
Taste	7.53	8.20	7.53	8.20	7.50	8.15	7.43	8.06	7.13	7.83	6.99	7.70	6.87	7.67
OAA	7.962	8.144	7.964	8.14	7.932	8.084	7.862	8.008	7.746	7.91	7.66	7.82	7.584	7.774
Total Score	47.772	48.864	47.784	48.84	47.592	48.504	47.172	48.048	46.476	47.46	45.96	46.92	45.504	46.644

FC- Fermented control, T3W– Wheat bran in T3

4.9.2.3.4. Organoleptic qualities of skimmed milk powder added fermented food mixture on storage

As revealed in Table 161, the mean score for appearance of FC and T_3SK was 8.33 initially, which decreased on storage and after six months, the score was 8.10 and 8.11 respectively. With respect to colour, FC and T_3SK had a mean score 8.54 and 8.56 respectively, which on storage decreased to 8.34 and 8.36 after six months. The flavour of FC and T_3SK was 7.10 and 7.50 initially and both in FC and T_3SK, there was a reduction in the flavour. By the end of the storage period, the mean score for flavour in FC and T_3SK was 6.61 and 7.13 respectively. The texture of FC and T_3SK was 8.31 and 8.32 initially and decreased on storage to 8.00 and 8.02 respectively. With regard to taste, T_3SK had a high score of 8.47 initially which decreased on storage to 7.80. The mean score for taste of FC was less (7.53) compared to T_3SK which decreased on storage and after six months the mean score was 6.87 in FC. Overall acceptability was also higher in T_3SK, the mean score varying from initial value of 8.236 to 7.888 after six months and this was 7.962 and 7.588 in FC.

4.10. Quantity of food mixtures recommended for daily use based on the viable count of *L. acidophilus*.

Table 162. Viable count of *L. acidophilus* in the developed food mixtures at the expiry period (after six months).

Treatment	Viable count of *L. acidophilus*	
	(x 10^7 cfu/g)	(x 10^7 cfu/5g)
T_1	95	475
T_3	133	665
T_8	210	600
T_3S	208	1040
T_3SB	134	670
T_3W	189	945
T_3SK	194	970

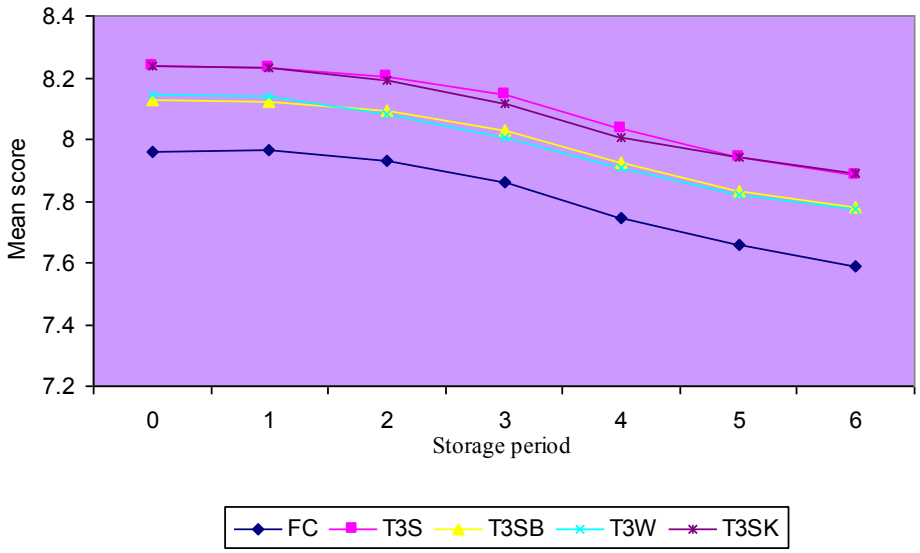


Fig. 45. Organoleptic qualities of the modified food mixtures on storage

Table 161. Mean score for organoleptic qualities of skim milk powder added fermented food mixture and fermented control on storage.

Quality attributes	Storage period in months													
	Initial		1		2		3		4		5		6	
	FC	T3SK	FC	T3SK	FC	T3SK	FC	T3SK	FC	T3SK	FC	T3SK	FC	T3SK
Appearance	8.33	8.33	8.33	8.32	8.31	8.30	8.27	8.27	8.20	8.19	8.15	8.15	8.10	8.11
Colour	8.54	8.56	8.55	8.57	8.53	8.54	8.48	8.50	8.44	8.45	8.40	8.41	8.36	8.38
Flavour	7.10	7.50	7.10	7.50	7.03	7.40	6.86	7.32	6.80	7.26	6.70	7.21	6.61	7.13
Texture	8.31	8.32	8.31	8.32	8.29	8.32	8.27	8.27	8.16	8.17	8.06	8.07	8.00	8.02
Taste	7.53	8.47	7.53	8.46	7.50	8.39	7.43	8.23	7.13	7.96	6.99	7.88	6.87	7.80
OAA	7.962	8.236	7.964	8.234	7.932	8.19	7.862	8.118	7.746	8.006	7.66	7.944	7.588	7.888
Total Score	47.772	49.416	47.784	49.404	47.592	49.14	47.172	48.708	46.476	48.036	45.96	47.664	45.528	47.328

FC- Fermented control, T3SK– Skim milk powder in T3

It is essential that products sold with any health claims meet the criterion of a minimum of 10^6 cfu/g probiotic bacteria at the expiry date, because the minimum therapeutic dose per day is suggested to be 10^8- 10^9 cells (Kurmann and Rasic, 1991).

As revealed Table 162, the food mixtures showed a desirable level of 10^7 cfu even in one gram of the food mixture after six months of storage. Hence the dose was fixed as five grams per day to assure for the functional benefits of *L. acidophilus* in the GI system.

Since *L. acidophilus* fermented food mixtures were slightly acidic in taste, it goes well with acidic foods like buttermilk, fruit juices etc. It can also be taken with lukewarm water or milk according to the availability, but the temperature should be below 45°C. Since a clinical trial has not been conducted in the present study, the developed food mixtures can be regularly used as a functional food supplement in improving the general health status.

4.11. Cost of the developed food mixtures

Table 163. Cost of the developed food mixtures (per 400 g)

Treatment	Fixed cost (in Rs) (cost of machinery/depreciation/ labour cost)	Cost of raw materials (in Rs per kg)	Total cost (in Rs)
T_1	1250	96.30	538.52
T_3	1250	84.40	533.76
T_8	1250	88.00	535.20
T_3S	1250	68.83	527.53
T_3SB	1250	122.23	548.89
T_3W	1250	69.08	527.63
T_3SK	1250	72.33	528.93

As per Table 163, in all the food mixtures, the cost of machinery, depreciation and labour cost was fixed and changes were only in the proportion of raw materials. Hence price variation was observed only in the raw materials used. The cost of the developed food mixtures ranged between Rs 530 to Rs 550 per 400 gram.

Discussion

5. DISCUSSION

The results of the study entitled "Standardisation and quality evaluation of banana based probiotic fermented food mixtures" are discussed in this chapter.

5.1. Probiotic characteristics of *L.acidophilus* MTCC 447.

Probiotics are defined as live microbial supplement that beneficially affects the host animal by improving its intestinal microbial balance. To provide health benefits, probiotics must overcome physical and chemical barriers such as acid and bile in the GI tract and also should exhibit antimicrobial properties. Acid and bile tolerance and antimicrobial activity of *L.acidophilus* is strain dependent and hence these characteristics of the selected strain were studied.

5.1.1. Effect of pH on the survival of *L.acidophilus*

The survival of any microorganism in the stomach should be pH-HCl dependent (Giannella *et al.,* 1972). In the present study the viable count of the selected strains of *L.acidophilus* varied at different pH from 1.5 to 9.0.which was similar to the results of earlier researchers who reported that the survival rate of lactic acid bacteria at different pH levels varied depending on species and strain involved (Conway *et al.*, 1987 and Ballongue, 1993).Viability was shown even at low pH of 2.0 (27×10^{9} cfu/ml). In the present study no viable cells were found at pH 1.5, which is in line with the findings of Bolin *et al* (1997). Maximum viability of 8.9×10^{9}cfu/ml was at pH 3.5.

Rashid *et al*. (2007) observed that *Lactobacillus delbruecki* exhibited maximum viability at a wide range of pH (3.0 to 5.0) and *L. acidophilus* grows readily at rather low pH values (below pH 5.0). In the present study also maximum survival of *L.acidophilus* was observed at pH values below 5.0. Borpuzari *et al.* (2007) revealed that different species of *L.acidophilus* were able to grow at pH 3.0 and showed gradual

increase in their population upto pH 7.0 and thereafter with an increase in pH of the growth medium, a fall in the total viable count was recorded.

The strains used in this study showed a growth at pH 2.0 and showed gradual increase in their population upto pH 4.5 with maximum population at pH 3.5. Earlier studies by Liong and Shah (2005) in 11 strains of *Lactobacillus* revealed that all the strains showed tolerance to pH 2.0 for two hours despite variations in the degree of viability. He also found that *L. acidophilus* was the most acid tolerant strain with more than 10^7 cfu/ml after incubation for two hours at pH 2.0.

5.1.2. Effect of bile acids on the survival of *L acidophilus*

Bile acid tolerance is an essential criterion for probiotic organism for colonisation in the colon (Huis and Havenaar, 1992).

An increased reduction in the viability of *L acidophilus* strain with the time of incubation with a bile salt concentration of 1-2 per cent was observed which is in line with the findings of Sridar *et al*. (2003).At higher bile salt concentration of 3-4 per cent, viability was observed for one hour incubation which was similar to the findings of Gilliland and Walker (1990) and Lin *et al*. (1991) who reported that *L.acidophilus* NCFM strains were capable of growing in bile concentration upto 3 per cent for one hour incubation.

Lactobacillus acidophilus are mostly delivered in a food system and must be acid and bile tolerant to survive in the GI tract. The time from entrance to release from the stomach has been estimated to be approximately 90 minutes, with further digestive processes requiring longer resistance time (Berada *et al.,* 1991).Stresses to organism begin in the stomach, with pH between 1.5 and 3.0 and in the upper intestine that contain bile (Lankaputhra and Shah, 1995; Corzo and Gilliland, 1999).Survival at pH 3.0 for two hours and at a bile concentration of 1000 mg/l is considered optimal acid

and bile tolerance for probiotic strain (Usman and Hosono, 1999). Hence *L.acidophilus* MTCC 447 can be considered as an acid and bile tolerant probiotic strain.

5.1.3. Antibacterial activity of *L.acidophilus* (MTCC 447)

Most probiotic strains are believed to have an ability to colonise the intestinal tract and thereby positively affect the mircoflora and perhaps exclude colonization by pathogens. *L.acidophilus* MTCC 447 has also exhibited an antagonistic activity against some enterophathogens at different pH levels. *Salmonella enteritidis* was the maximum inhibited pathogen with a well diameter of 24 mm at pH 3.0 and 3.5. This organism was maximum inhibited at all pH levels except at pH 7.0. A similar study conducted by Khedkar *et al.* (1998) also revealed the antibacterial effect of *Bifidobacterium adolescentis* against *Staphylococcus aureus, Salmonella typhosa, Shigella sonnet* and *Plesiomonas aeruginosa*. He also reported that an increase in pH from 3.8 to 4.8 affected the antibacterial activity as indicated by the reduced zone of inhibition from 17 to 10mm. At pH 5.0, the activity was completely lost. A similar effect of pH on the antibacterial activity elaborated by probiotic organism was reported in earlier studies by Anand *et al.* (1984) and Kang *et al.* (1989). Inhibitory effect of *L.acidophilus* on enteropathogens isolated from food was also studied by Singh *et al.* (2004). He also revealed that *Lactobacillus* strain 1 was the most effective to inhibit the growth of 35 strains out of 75, while *Lactobacillus- R* and *Lactobacillus*-3 could inhibit growth of only 30 and 19 strains respectively. None of the lactobacillus inhibited *Proteus mirabilis, Morganella morganii* and *Plesiomonas shigelloides* strains.

Similar results were also obtained by Borpuzari *et al.* (2007) who studied the antibacterial characteristics of *L. acidophilus* strains isolated from fermented milk. They isolated 10 strains of *L. acidophilus* and all the 10 strains exhibited antibacterial activity against *E.coli*, maximum being shown by strain *L. acidophilus* E_{30} (10mm) and the least (7mm) was exhibited by *L. acidophilus* H_{52}. The difference in the spectrum of antibacterial activity was found to be strain specific.

This difference in the antibacterial activity against enteropathogens might be due to the activity of a particular antibacterial substance synthesised by a strain and partly may be due to the presence of appropriate receptor site in the cell walls of the susceptible organisms. In the present study *L.acidphilus* MTCC 447 could not inhibit the growth of *Shigella flexneri.*

Liong and Shah (2005) had indicated that *lactobacillu*s can be beneficial in food products because of their ability to produce hydrogen peroxide. This hydrogen peroxide produced enabled them to suppress the growth of S.*aureus, E.coli, C.botulinum* and other undesirable microorganisms. In the present study, *L.acidophilus* MTCC 447 also inhibited *E.coli* which showed a diameter of 20mm at 3.0 pH but at 3.5 pH *Bacillus cereus* showed more inhibition than *E.coli.*

Earlier studies by Gupta *et al.* (1996) and Gilliland and Speck (1977) have also shown the inhibitory activity of *L.acidophilus* on common intestinal and food borne pathogens such as *Listeria monocytogenes, Salmonella spp, E. coli, Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium, Yersinia enterocolitica* and *Clostridium perfringens.* The inhibitory effect is thought to be brought out by either due to competition for nutrients or due to presence of starter derived inhibitors such as diacetyl, lactic acid, hydrogen peroxide and bacteriocins (Iwana and Masuda, 1990 and Abee., 1995). By competitive colonization, probiotic bacteria inhibit the adhesion of gastrointestinal pathogens to the intestinal mucosa (Conway, 1996). Studies have reported that *L.acidophilus* inhibits the pathogenic flora by production of certain antibiotics like Acidophilin, Lactocidin, Acidolin and Lactolin (Goldin, 1998). The strains vary in their ability to produce these substances and cultural conditions will influence the amount produced (Salminen *et al*., 1998). Organic acetic and lactic acids which are produced by lactic acid bacteria will lower intestinal pH and thereby inhibit the growth of many bacteria, especially pathogenic gram-negative types**.** These organic acids also increase peristalsis, thereby indirectly removing pathogens by accelerating their rate of transit through the intestine (Laroia and Martin ,1990).

Hydrogen peroxide produced by *lactobacilli* (Gilliland and Speck, 1977) may function through the lactoperoxidase-thiocyanate system, in which hydrogen peroxide

oxidizes the thiocyanate to release hydrocyanic acid, which is detrimental to food-borne pathogens. Carbon dioxide and diacetyl synthesized by lactic acid bacteria inhibit growth of pathogens (Mishra and Lambert,1996). Numerous bacteriocins, such as nisin, lactobrevin, acidophilin, acidolin, lactobacillin, lactocidin and lactolin, have been reported to be produced by *lactobacilli* (Shahani and Chandran, 1979). Bacteriocins are active against a wide range of food-borne pathogens, depending on their specificity (Mishra and Lambert,1996). Studies conducted by Coconnier *et al*. (2000) has also establishd the secretion of antimicrobial substances by *L.acidophilus* strain isolated from human gut.

Hydrochloric acid secreted by the gastric mucosa may kill many of the food-borne pathogens, as may both bile acids and pancreatic enzymes. The motility of the intestine, epithelial mucin secretion and the activity of microflora can act synergistically to kill pathogens and/or prevent their colonization and subsequent translocation across the intestinal mucosa. Several of these non-specific intestinal defence parameters may be modulated by diet.

5.2. Standardisation of ingredients in the food mixtures

Food stuffs containing probiotics have been considered to be beneficial to health for many years but only to recent years there has been scientific support for these beliefs. A number of food manufacturers in the world are exploiting the commercial opportunities for such foods. Combining probiotics and prebiotics called a 'synbiotic' could beneficially affect the host by improving survival and implantation of live dietary supplements in the gastro-intestinal flora by selectively stimulating the growth or activating the catabolism of one or a limited number of health promoting bacteria in the intestinal tract and by improving gastro-intestinal tract's microbial balance.

The foods selected for developing the probiotically fermented food mixtures were banana flour, defatted soya flour, green gram flour, ripe mango, papaya and tomato. From the 56 combinations tried, 14 fermented food mixtures with *Lactobacillus*

acidophilus MTCC 447 were selected based on organoleptic qualities like appearance, colour, flavour, texture and taste. All the selected food mixtures contained 60-70 percent banana flour as the major constituent and 20 percent of either defatted soya flour or green gram flour. Fruit pulps viz mango, papaya and tomato either singly or in combination were present in 10-20 percent levels in all the acceptable combinations.

Products combining *Lactobacillus acidophilus* (as probiotic) and certain fruits such as banana (as prebiotic) that might provide functional benefits (as synbiotic) have been suggested and studied by Prajapathi *et al*. (1987). Banana possesses high contents of sugars mainly sucrose, glucose and fructose and is suitable for microbial fermentation (Vega *et al.,* 1988).

Studies by Saarela *et al*. (2002) indicated that soya is a good substrate for probiotic bacteria. Fruit juices have also been suggested as a good medium for probiotic ingredients. Juices fortified with *Lactobacillus rhamnosus GG* exhibited good shelf life and acceptable sensory properties after seven days of storage (Heller, 2001). Rani and Khetarpaul (1999) developed a probiotic fermented food mixture that is, RSMT containing freshly ground rice, defatted soya flour, skimmed milk powder and fresh tomato pulp (2:1:1:1 w/w) with good acceptability. Studies conducted by Babu *et al*., (1992) also proved tomato and papaya pulp as good substrates for *Lactobacillus acidophilus*. In another similar study by Sindhu and Khetarpaul (2004); barley flour, milk coprecipitate, green gram paste and tomato pulp (2:1:1:1 w/w) were used for *Lactobacillus acidophilus* fermentation, and the food mixtures were found to be organoleptically acceptable to human palate and maintained adequate cell viability.

Semjonovs *et al.* (2008) had stated that, lactic acid bacteria fermented products are well known for their sensory qualities throughout the world and also for their taste, nutritive value and therapeutic properties. In the present study also, 14 substrates with different combinations of selected foods fermented with *Lactobacillus acidophilus* were identified based on maximum organoleptic qualities.

5.3. Optimisation of variables for probiotic fermentation of food mixtures with *Lactobacillus acidophilus* MTCC 447

Production of a good probiotic food mixture with desirable viable counts require optimization of viable cell count, for this is dependent on multiple factors which are usually strain specific. Several earlier studies have optimised fermentations which have generally focused on the effects of pH, temperature and composition of culture medium. In the present study, total viable count in the product was maximised while variables like substrate concentration (food mixture), quantity of the inoculum (*Lactobacillus acidophilus*), time of incubation, pH and temperature were kept at acceptable levels.

As revealed in the study, in all the 14 treatments, 25g of the substrate produced maximum viable counts of *Lactobacillus acidophilus* ranging from 39-76 $x10^7$ cfu/g after fermentation and freeze drying. It was found that by increasing the substrate concentration to 50g, there was a decline in cell count ranging from 28-58cfu/g($x10^7$). Similarly maximum viable counts in the products were obtained at 4.5 pH the maximum being in T_6 (103 $x10^7$cfu/g). When the pH of the medium was increased to 5.5, again there observed a decline in viable count ranging from 27-61cfu/g($x10^7$) and even too few colonies to count in T_2, T_5 and T_7.Temperature for fermentation of food mixture was optimised at 37°C for all treatments, producing maximum viable counts ranging from 37-99 $x10^7$cfu/g. Time of incubation with maximum cell count was 24 hours with an inoculum concentration of 300µl.

Thus for all the treatments, fermentation with 25g of the food mixtures at pH 4.5, inoculated with 300µl ($119x10^6$cfu/ml) and incubated at 37°C for 24 hours gave the maximum total viable counts of *Lactobacillus acidophilus* MTCC 447 ranging from 9.13 to 9.45 log cfu/g which is far above the desired level of probiotic organisms as recommended by Shah *et al*. (1995)

Earlier, similar optimization studies were conducted by Santos and Soccol (2003) in the development of a probiotic beverage using cassava flour and strains of *Lactobacillus casei* and *Lactobacillus acidophilus*. The optimised parameters were

temperature of incubation of 35°C, fermentation time of 16 hours, cassava flour concentration of 20 per cent and inoculum rate of four per cent for *Lactobacillus casei* and 4 per cent for *Lactobacillus acidophilus.*

Liong and Shah (2005) had also optimised cholesterol removal by *Lactobacillus casei ASCC 292* in presence of six types of prebiotics in the substrate viz sorbitol, mannitol, maltodextrin, high amylase maize, inulin and fructo oligo saccharide (FOS). He revealed that a combination of *Lactobacillus casei ASCC 292,* FOS and maltodextrin was most efficient for removal of cholesterol.

An optimization study was conducted by Angelov *et al*., (2006) in developing a synbiotic functional drink from oats by combining a probiotic starter culture and whole grain oats substrate. In this study oat mash was inoculated with 1.0, 5.0 and 10 per cent starter culture suspension of *Lactobacillus plantarum B 28,* aiming to achieve the required levels of viable cells in probiotic products. With 5.0 and 10 per cent inoculum concentration applied, in six hours, the levels of viable cell count reached $9.3x10^9$ and $7.5x10^{10}$cfu/ml and hence selected 5.0 per cent starter culture concentration for product development.

Recently, an optimization study was conducted by Harbinder *et al*., (2009) to find the effect of yoghurt bacteria and probiotic culture on the textural characteristics of mango soy fortified probiotic yoghurt (MSFPY). The optimization of culture addition was done with yoghurt bacteria (*Streptococcus thermophilus)* and probiotic culture *(Lactobacillus acidophilus)*. Substrate was made with 78.3 per cent toned milk, 14.5 per cent soymilk and 7.2 per cent mango pulp. The study revealed that the mean optimum culture addition rate of 1.43 (0.7 per cent) *Streptococcus thermophilus* and 1.51 (0.75 per cent) *Lactobacillus acidophilus*. *Lactobacillus acidophilus* was recommended for yielding an acceptable and good quality MSFPY.

Thus optimization of variables in probiotic fermentation in relation to the processes and expected final product, is a useful tool to differentiate the probiotic fermented foods and thereby expand the range of products to satisfy the heterogeneous

consumer demand. The data achieved by optimization in the present study also suggested a strong effect of independent variables on the viable cell count in the final product, by fermenting with *Lactobacillus acidophilus MTCC 447.*

5.4. Development of food mixtures

Microorganisms are found to be associated with a variety of foods we consume. They bring about changes in the quality of the food, causing spoilage and decomposition. Pathogenic microorganisms present in food cause disease when consumed. Hence, control of pathogens in foods is a major concern. During food preparation and preservation, it is necessary to eliminate the pathogens and spoilage organisms. Major factors important in the selection of proper food preservation technique include the characteristics of the concerned microbes, consumer perception and acceptance of preservation methods as well as their impact on the quality of food, safety and cost.

Autoclaving is a standard procedure for sterilizing the medium for bacterial culture. In the present study, since the medium is the food mixture, it has to be autoclaved for the growth of the selected strain of *L. acidophilus*. Autoclaving involves high temperature under moist condition which can bring about certain chemical changes in the nutrients as well as changes in the colour, texture and flavour affecting the overall acceptability of the product. Many strains of *L.acidophilus* which are used for food fermentation are known to produce certain antagonistic compounds called bacteriocins. These inhibit spoilage bacteria and also some of the pathogenic microorganisms. Hence, in order to study the feasibility of avoiding autoclaving in the preparation of food mixtures and also to study the effect of the selected strain of *L.acidophilus* in inhibiting the growth of contaminating miroorgansims in unautoclaved media, a fermented control without autoclaving the food mixtures was also prepared, along with autoclaved fermented food mixture (UFFM). But the unautocalved fermented food mixtures were heavily contaminated. Sometimes, this may be due to the production of

bacteriocin in very low concentration in unautocalved food mixtures to inhibit the pathogens already present in the food system.

5.5. Quality evaluation of the food mixtures

Lactobacilli, having probiotic properties play an important role in the production of various wholesome foods. This organism may not only change the microbial composition of the intestinal tract when ingested along with food, but may also bring some desirable changes in the colour, flavour, taste and nutritional composition of the products.

In the present study, chemical composition of the 14 fermented food mixtures (FFM) prepared under optimum conditions were studied along with their respective control of unfermented samples (UFFM).

Moisture in fermented and unfermented food mixtures

Moisture content in a flour is very important regarding its shelf life, lower the flour moisture, the better its storage stability.

Moisture content in fermented food mixtures varied from 1.81 to 1.86 g/100g. The moisture content in T_1 (1.80 g/100g), T_5 (1.81 g/100g), T_{12} (1.82 g/100g) and T_{13} (1.83 g/100g) were significantly low when compared to other FFM. In the unfermented samples also, the moisture content varied from 1.81 to 1.85 g/100g. There was no significant difference in the moisture content of fermented and unfermented food mixtures. This result is in agreement with those of previous works by Sindhu and Khetarpaul (2004), who observed no change in the moisture content due to probiotic fermentation of BCGT food mixture containing barley flour, milk coprecipitate, sprouted green gram and tomato pulp.

The findings are also in agreement with the results of Goyal and Khetarpaul (1994) and Sharma and Khetarpaul (1997) who also reported no difference in the moisture content of probiotic fermented as well as unfermented food mixtures.

As stated by Tsen (2007) the freeze-dried fermented products exhibited little hygroscopicity because of the consumption of monosaccharides by the fermenting organisms.

Titrable acidity in fermented and unfermented food mixtures

Titrable acidity in fermented foods varied from 2.50 to 2.68 g lactic acid/100g, which showed no significant variation between the treatments. Titrable acidity was significantly high in probiotic fermented foods. In UFFM, titrable acidity varied from 1.23 to 1.34 g lactic acid /100g.

During fermentation, probiotic organisms convert glucose to lactic acid which is responsible for the increase in titrable acidity in the fermented food products. Production of organic acids such as lactic acid and acetic acid by *L. acidophilus* are reported by Laroia and Martin (1990) which lowers intestinal pH and thereby inhibits the growth of pathogens (Kohajdova and Karovicova, 2007). Because of this property, *L. acidophilus* fermented food products have been recommended as a dietary adjunct. Johnson *et al* (1987) suggested that, strain that could produce sufficient acid with desirable traits should be used for the manufacture of *acidophilus* products.

Similar results which showed an increase in titrable acidity has been reported in fermented cereals and legumes (Agte *et al*., 1997 and Urga *et al*., 1997) and *L .acidophilus* fermented food mixtures containing cereal, legume, skim milk powder and fresh tomato pulp (Rani and Khetarpaul, 1998).The results of the study conducted by Chavan *et al*. (1998) also revealed an increase in the titrable acidity of Sorghum-green gram blend (70:30, w/w) during fermentation.

Sindhu and Khetarpaul (2001) also had a similar observation of increased titrable acidity in the probiotic fermentation of indigenous food mixture containing tomato pulp using *L. casei* and *L. plantarum*. Similar findings were also reported by Sindhu and Khetarpaul (2004), who observed an increase in titrable acidity from 1.78 to 2.93 g lactic acid / 100 g in BCGT food mixtures fermented with probiotic organisms such as *S .boulardii and L. casei.*

Protein in fermented and unfermented food mixtures

There was a significant variation in the protein content of fermented food mixtures. Maximum protein was observed in T_3 (9.81 g /100g) and the least in T_{11} (5.98 g/100g). Their corresponding control values also showed the maximum in T_3 (7.65 g/ 100g) and minimum in T_{11} (4.07 g/ 100g). Protein content in the fermented food mixtures were significantly high. Increased protein content due to fermentation has been reported in barley (Ashenafi and Mehari, 1995) and ragi (Basappa *et al*., 1997). Wang (2007) also revealed an increase in the content of crude protein in pea nut flour fermented with *L. plantarum* P9.

Onimawo *et al.* (2003) also found that the unfermented pumpkin seeds contained 28.0% crude protein and on fermentation the protein content increased significantly to 39.4%. Similarly a significant increase ($P < 0.05$) in the protein content (21.1 per cent) of the cassava peels fermented with *Lactobacillus delbruckii, Lactobacillus coryneformis* and *Saccharomyces cerevisae*(2:1:1) was also reported by Oboh (2006).

Lentil (Lens culinaris var. vulgaris) flour naturally fermented for 4 days at 28°C and 79g/l concentration caused a slight increase in total protein (Tabera *et al*., 1995). Wet-cooked sorghum flour inoculated with lactic bacteria (*L. fermentum*, *L.bulgaricus*, *L.lactis*, *Pediococcus pentosaceus* and *Pediococcus cerevisiae*) and a mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* showed an

increase in titratable acidity, free amino acids and total protein content. (Correia *et al*., 2004).

Peanut flour fermented with *L. plantarum* P9 showed an increase in the content of crude protein and also in the degree of protein hydrolysis (Wang, 2007). Adejuyitan *et al.* (2009) in their study reported that the flour samples from fermented tigernuts contained higher amounts of protein than the unfermented flour. The protein content increased with fermentation period. At 0 h fermentation, the protein content of the flour was 6.67 per cent while for 24, 48, and 72 h fermentation, the flour contained 7.73, 8.40 and 9.23 per cent respectively.

As stated by Zamora and Fields (1979) the increase in protein content can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles.

According to Oboh and Akindahunsi (2003) the increase in the protein content of the fermented food mixtures could be due to the possible secretion of some extracellular enzymes (proteins) such as amylases, linamarase and cellulase into the food mixture by the fermenting organisms in an attempt to make use of the starch as a source of carbon.

On the contrary a few others (Sindhu and Khetharpaul, 2004 and Rani *et al.,* 1996) reported a reduction in crude protein content of cereal – legume based probiotic fermented food mixtures.

β carotene in fermented and unfermented food mixtures

There was a significant variation in the β carotene content of FFM, maximum being in T_1 (563.52 µg/100g) and the least in T_{11} (283.66). The corresponding control of T_1 and T_{11} showed a slightly higher value for β carotene but the variation observed was not significant.This result is in line with the findings of Li *et al*. (2007) who reported no

significant variation in the β carotene content of fermented and unfermented maize porridges.

In the present study the high β carotene content in food mixtures may be due to the carotene rich fruits like mango, papaya and tomato with minimum loss due to freeze drying.

Fibre content in fermented and unfermented food mixtures

None of the fermented food samples contained detectable amount of crude fibre, whereas crude fibre content in unfermented food mixtures varied from 0.31 to 0.83 g /100g. The decrease may be due to solubilisation of fibre by microbial enzymes.This result is in accordance with those reported for cereal based food mixtures fermented with *L. acidophilus* (Rani *et. al.* 1996 and Sindhu and Khetharpaul, 2004)

Raimbault and Tewe (2001) observed that during fermentation, carbohydrate including cellulose, pectin, lignocellulose and starch are broken down by fermenting microorganisms thereby reducing the fibre content of such foods.

TSS in fermented and unfermented food mixtures.

TSS in fermented food mixtures ranged from 11.61 to 11.68°brix, which was significantly low than that of unfermented food mixtures (14.863 to 14.973°brix) There was no significant variations in the TSS content of fermented food mixtures , but a significant difference was observed in unfermented food mixtures. This finding is in line with the results of Steinkraus (1995) where the initial TSS content in salivated maize flour extract was 18.6 per cent, which fell to 4.6 per cent as the fermentation was completed. Similar finding were also reported of Namugumya and Muyanja (2009) who developed a fermented beverage Kwete, and showed a decrease in TSS from 9.02 to 5.87 after 72h fermentation. During fermentation, the metabolic activities of lactic acid bacteria may lead to the production of lactic acid from the break down of hexoses and

pentoses (Adams and Moss, 2008). This explains the increase in the titratable acidity and decrease in the total soluble solids during fermentation.

Starch in fermented and unfermented food mixtures

Starch was significantly low in fermented food mixtures which varied from 51.93 to 55.06 g/100g as against 61.337 to 66.943 g /100g in unfermented food mixtures. A similar decrease in starch content was observed by Sotomayor *et al*. (1999) during fermentation of lentil flour.

Fermentation using endogenous grain microflora at 30 °C on the primary nutrients in finger millet *(Eleusine coracana)* also showed a decrease in the starch and long-chain fatty acid content. **(**Antony *et al*., 1996**).** The starch content in cassava flour was 76.86 per cent which on fermentation with *L.plantarum* to prepare fufu reduced the starch content to 70.72 per cent (Sobowale *et al*., 2007). The decrease in starch content caused by fermentation could be attributed to the microbial amylose which breaks down starch to fermentable sugars. (Peterson, 1971).

Reducing and total Sugar in fermented and unfermented food mixtures

In the present study, reducing sugars and total sugars were also significantly low ($p<0.05$) in fermented food samples. Reducing sugars varied from 2.11 to 3.07 g/100g as against 3.17 to 4.96 g/100g in UFFM and total sugar varied from 4.76 to 5.64 in FFM as against 9.023 to 9.967 g/100g in UFFM.

Yoon *et al*. (2004) in a similar study observed that, lactic acid cultures rapidly fermented tomato juice and reduced the level of total sugar. *L.plantarum* reduced the sugar level from an initial value of 32.4 mg/ml to 25.2, 21.0 and 19.3mg/ml after 24, 48 and 72 h fermentation, respectively**.** The sugar content in cassava flour was 5.21 per cent which on fermentation with *L.plantarum* reduced to 4.41 to 4.60 per cent (Sobowale *et al*. 2007). An indigenous food mixture developed by mixing rice flour,

whey, sprouted green gram paste and tomato pulp fermented with a mixed culture of *L. casie*, *S. boulardii* and *L. plantarum* also showed a significant ($p < 0.05$) reduction in total sugars, reducing sugars and starch content (Sindhu *et al.*, 2005).

The microbes break fermentable sugars, into simpler substances, such as carbon dioxide and alcohol. Because these simple substances are toxic to food-spoiling microbes, they act as natural preservatives in the food system.

Minerals in fermented and unfermented food mixtures

In the present study there was no significant difference in the calcium and potassium content of fermented and unfermented samples. There was a significant increase in the iron content of FFM than UFFM. A similar result was reported by Sharma and Khetarpaul (1997) regarding the mineral content in fermented foods. Rice-dehulled blackgram blends developed and fermented with whey at 35 °C for 18 h did not significantly change the total amount of calcium, phosphorus, and iron present in the blends. On the other hand, the HCl-extractability of calcium, phosphorus, and iron was enhanced considerably after whey incorporation and fermentation of cereal-legume blends.

As stated by Jood and Khetarpaul (2005) reduction in antinutrients due to fermentation may increase the bioavailability of various minerals but there need not be any change in the total mineral content in fermented foods.

Thiamine and riboflavin in fermented and unfermented food mixtures.

The thiamine and riboflavin content increased significantly in fermented food mixtures compared to unfermented food mixtures. A similar result was observed by Deeth and Tomine (1981) where lactic acid bacteria have been shown to increase the content of the B vitamins in fermented foods.

Similarly, fermentation of food with lactic acid bacteria has been shown to increase niacin and riboflavin levels in yoghurt, vitamin B_{12} in cottage cheese and vitamin B6 in cheddar cheese (Shahani and Chandran, 1979 and Alm, 1982). Increased amounts of riboflavin, thiamine and lysine due to the action of *L. acidophilus* in fermented blends of cereals were also reported by Hamad and Fields (1979).

Imitation milk obtained from the seeds of the groundnut (*Arachis hypogaea* L.) fermented with a culture pack consisting of a mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* to obtain a yoghurt-like product recorded an increase in the niacin, riboflavin and thiamine (Sunny *et al.,* 2004).

***In vitro* digestibility of starch and protein in fermented and unfermented food mixtures**

Fermentation of food is an important method which significantly lowers the content of antinutrients and thereby improves the nutritive value of foods. Fermentation encourages the multiplication of selected organisms and their metabolic activities in food. If fermentation is carried out with probiotic organisms, it might have specific added advantages apart from improvement of nutritive value. In addition to nutrient synthesis, probiotic may improve the digestibility of some dietary nutrients such as carbohydrates, proteins and fats.

In the present study also, fermentation of food mixtures with lactic acid bacteria has been shown to increase the digestibility of starch and protein.

The *in vitro* starch digestibility of unfermented food mixtures ranged between 54.41 to 56.34 per cent and this improved significantly upon fermentation to 78.57 to 83.60 per cent. Similar findings were reported by Rani and Khetarpual (1999) where the starch digestibility of unfermented autoclaved RSMT mixture was 62.65 per cent which on fermentation improved to 78.33 per cent. It was reported that probiotic fermentation of indigenous food mixtures containing tomato pulp using *L. casie* and *L. plantarum*

showed an improvement of the digestibilities of starch and protein (Sindhu and Khetarpaul, 2001).

The increase in starch digestibility of fermented products may be related to enzymatic properties of microbes, which ferment the substrate. The fermenting micro flora brings about the breakdown of starch to oligosaccarides. The enzymes bring about the cleavage of amylose and amylopectin to maltose and glucose. The presence of α amylase in the fermenting bacteria was noticed by Bernfeld (1962) and Soni and Sandhu (1990). Complete elimination of alpha-amylase inhibitors in most fermentation also contributes to improved starch digestibility.

The IVPD in the unfermented food mixtures ranged between, 57.15 to 57.87 percent which significantly increased on fermentation to 85.41 to 86.21 per cent. A significant difference between the protein digestibility of fermented and unfermented food mixtures was noted which was similar to the findings of Rani and Khetarpaul (1999). An increase in protein digestibility was also observed by Chavan *et al*. (1998) in sorghum-green gram blend after fermentation. Enhanced protein digestibility after fermentation has been reported in cereal-legume-whey blends (Sharma and Khetarpaul, 1997).

Indigenously developed RWGT food mixture which contained rice flour, whey, sprouted green gram paste and tomato pulp (2:1:1:1 w/w) fermented with 2% liquid culture (containing 10^6 cells/ml broth of *L. casei* and *L. plantarum*) showed a drastic reduction in the contents of phytic acid, polyphenols and trypsin inhibitor activity while significantly improving the *in vitro* digestibilities of starch and protein. Sequential culture fermentations brought about higher changes as compared to single culture fermentations (Sindhu and Khetarpaul, 2002). Food mixture which contained barley flour, milk coprecipitate, sprouted green gram paste and tomato pulp and fermented with *S. boulardi* and *L. casei* resulted in maximum increase in starch digestibility by 96 per cent (Sindhu and Khetarpaul, 2001), and protein digestibility by 50 per cent.

The improvement in protein digestibility is mainly associated with the enhanced metabolic activity of the fermenting organisms (Hesseltine, 1983). An improvement in protein digestibility of fermented products is mainly associated with an enhanced proteolytic activity of the fermenting microflora. High proteinase activity has been reported by various workers in fermented protein foods (Wang and Hasseltine, 1981; Odunfa, 1985). The improvement in IVPD caused by fermentation could be attributed to the partial degradation of complex storage proteins to more simple and soluble products (Chavan *et al*., 1988); it could also be attributed to the degradation of tannins, polyphenols and phytic acid by microbial enzymes.The increase in digestibility may also be due to reduced antinutrient content of the fermented foods as antinutrients are known to inhibit amylosis and proteolytic activity (Jood and Khetarpual, 2005). An increase in amino nitrogen by fermentation signifies partial breakdown of protein to peptides and amino acids, resulting in improved protein digestibility (Kao and Robinson, 1978; El Hag *et al.,* 2002).

Viability of *L. acidophilus* in the food mixtures

Viability and activity of the probiotic bacteria are important considerations, because the bacteria must survive in the food during shelf life and during transit through the acidic conditions of the stomach, and resist degradation by hydrolytic enzymes and bile salts in the small intestine (Playne, 1994).

To realize health benefits, probiotic bacteria must be viable and available at a high concentration, typically 10^{6} cfu/g of product (Shah 2001). Products sold with any health claims should meet the criterion of a minimum 10^{6} cfu/ml probiotic bacteria at the expiry date, because the minimum therapeutic dose per day is suggested to be 10^{8}–10^{9} cells (Kurmann and Rasic, 1991).

In the present study the maximum viable count was observed in T_6 (292 x 10^{7} cfu/g) and least in T_{13} (136 x 10^{7} cfu/g).The viability of *L .acidophilus* in the treatments

varied from 9.13 to 9.45 log cfu/g as against the desirable level as recommended by Shah *et al.* (1995)

Similar results were obtained by Sindhu and Khetarpaul (2001) in their BCGT (barley flour, milk coprecipitate, green gram and tomato pulp) food mixture. Single cell fermentation with *L.casei* resulted in a viable count of 9.88 log cfu/g and with *L.plantarum* showed a viable count of 9.11 cfu/g. Prajapathi *et al.* (1987) prepared a slurry using neutrallised acidophilus milk, banana paste, tomato juice concentrate and ground sugar at the rate of 40, 10 and 15 per cent respectively and observed a *Lactobacilli* count of 8.71 x 10^7 cfu/g.

Another similar study was by Angelov *et al*. (2006) who developed a new oat based probiotic drink fermented with lactic acid bacteria. He also observed a viable count of 9.3 x10^9 cfu/ml with 5 per cent inoculum concentration. Arora *et al.* (2008) developed two indigenous food mixtures by mixing raw and germinated pearl millet flour, whey powder and tomato pulp fermenting with *L. acidophilus* and found that the growth of *L. acidophoilus* was significantly higher (8.64 cfu/g) in germinated flour mixture. Yoon *et al.* (2004) studied the suitability of tomato juice as a raw material for the production of probiotic juice by lactic acid bacteria and observed a viable cell count of 10^8 cfu/ml after fermentation for 72 h at 30^{o}C. Wang *et al*. (2007) evaluated the probiotic value of peanut flour fermented with different strains of lactic acid bacteria and found *L. plantarum* P9 grew to the highest cell population (9.48 log cfu/g) in peanut flour after 72h fermentation at 37^{o}C. Ouwehand *et al.* (2004) developed a probiotic oat based cereal bar fermented with *B.lactis* Bb-12 and found a viable count of 5x10^9 cfu/ bar (25g).

Cell viability in probiotic foods depends on the strains used, interaction between species present, culture condition, oxygen content, final acidity of the product and the concentration of lactic acid and acetic acid in the food system. Bacterial viability is important because many clinical studies suggest that live bacteria are

mandatory to the beneficial effect of probiotic dietary supplements. In the present study all the 14 food mixtures showed a good viability of *L. acidophilus*.

However, several studies have shown that non viable probiotics can have beneficial effects such as immune modulation and carcinogen binding in the host (Ouwehand and Salminen, 1998; Salminen *et al*. 1998a). Thus for certain probiotic strain, it might be sufficient that they grow well during initial production steps (to obtain high enough cell numbers in the product) but they do not necessarily need to retain good viability during storage.

Several factors could affect the cell viability of lactic acid cultures in probiotic food products. Some products produced during lactic acid fermentation such as lactic acid, diacetyl, and acetaldehyde could be associated with the loss of viability of added probiotic bacteria (Post, 1996). Lactic acid starters are reported to produce bacteriocins against probiotic bacteria and vise versa (Dave and Shah, 1997).

Organoleptic qualities of the fermented and unfermented food mixtures.

The benefits of fermentation may include improvement in palatability and acceptability by developing improved flavours and textures. Lactic acid fermentation enhances considerably the sensory properties of food resulting in a variety of tastes (Nout and Ngoddy, 1997). During fermentation, lactic acid bacteria, yeast and other bacteria contribute significantly to flavour development (Oyewole, 1991). The cultures used in food fermentation are, however, also contributing secondary reactions to the formation of good flavour and texture (Hansen, 2002).

As per the findings of the present study, there was no significant difference in the appearance, colour and texture of fermented and unfermented food mixtures, but fermented mixtures had a significantly higher acceptability scores with regard to flavour, taste and overall acceptability

During fermentation, several volatile compounds are formed, which contribute to a complex blend of flavours in products. The presence of aromas represented by diacetyl, acetic acid and butyric acid makes fermented cereal-based products more appetizing (Blandino *et al*., 2003). The proteolytic activity of fermentation microorganisms often in combination with malt enzymes may produce precursors of flavour compounds, such as amino acids, which may be deaminated or decarboxylated to aldehydes and these may be oxidized to acids or reduced to alcohols (Mugula *et al*., 2003). However, the end product distribution of lactic acid fermentation depends also on the chemical composition of the substrate (carbohydrate content, presence of electron acceptors, nitrogen availability) and the environmental conditions (pH, temperature, aerobiosis/ anaerobiosis), controlling of which would allow specific fermentations to be channelled towards a more desirable product .

A probiotic fermented food made from pearl millet flour , chick pea flour , skim milk powder and fresh tomato pulp (2:1:1:1) with *L. acidophilus* (10^5 cells/ml) at 37 °C for 25 h showed good acceptability (Rani and Khetarpual, 1998).

The BCGT (Barley flour, milk coprecipitate, sprouted green gram paste and tomato pulp 2:1:1:1 w/w) probiotic food mixture developed by Sindhu and Khetarpual (2004) was found acceptable and the overall acceptability ranged from ‘like slightly’ to ‘like very much’. Dalev *et al*. (2006) developed a probiotic beverage based on cheese whey and soy with good sensory properties. Gupta *et al.* (1996) studied the organoleptic qualities of acidophilus yoghurt fermented with *L.acidophilus* 301 and normal yoghurt, and found no significant differences in the textural characteristics and both the products were almost identical with respect to colour, flavour, appearance, texture and overall acceptability with a score ranging from 7.4 to 7.8.

Fermentation has been used for centuries as means of improving the keeping quality of foods. Microorganisms by virtue of their metabolic activities, contribute to the development of sensory, shelf life and nutritional qualities. Factors related to the technological and sensory aspects of probiotic food production are of utmost

importance since only by satisfying the demands of the consumers can the food industry succeed in promoting the consumption of functional probiotic products.

5.6. Selection of the best six probiotic food mixtures.

The best six food mixtures selected based on nutritive value, organoleptic qualities and viable cell count were T_1, T_2, T_3, T_7, T_8 and T_9. All the selected six food mixtures had 60 -70 per cent banana flour, 20 per cent defatted soya flour and fruit pulps of mango and tomato.

Application of banana as a medium for lactobacillus fermentation has also been studied by Aegerter and Dunlap (1980) and De Porres *et al.* (1985).

Banana is said to posses high contents of sugars mainly sucrose, glucose and fructose which are suitable for microbial fermentation. Bananas are an exceptionally rich source of fructooligosaccharides (FOS), a group of compounds which have been shown to exhibit beneficial health effects by stimulating the growth of lactic acid bacteria in the colon by suppressing putrefactive pathogens. When fructooligosaccharides are fermented by these friendly bacteria, not only do numbers of probiotic bacteria increase, but so does the body's ability to absorb nutrients. The most common oligosaccharide in banana is inulin and its hydrolysates and oligofructans. The degree of oligosaccharide polymerisation is of importance. The major fraction in inulin has a degree of polymerisation of about 14. Because of the β configuration of the anomeric C-2 in their fraction monomers, inulin type fructans resist digestion in the upper GI tract. Thus it has been proposed that they may be called ‘colonic foods’- i.e. a food entering the colon and serving as a substrate for the endogenous bacteria thereby directly providing with energy and metabolic substrates. This might have contributed to the high viability of *L. acidophilus* in all the fermented food mixtures which contained 60 -70 per cent banana flour. Many patents concerning prebiotic oligosaccharides especially inulin compounds in banana have been claimed and this field is continuously increasing (Crittenden and Playne, 1996).

Rani and Khetarpaul (1999) developed a probiotic fermented food mixture RSMT, which contained defatted soya flour showing good acceptability and viability. Earlier studies conducted by Song *et al.* (2008) also reported that fermented soya bean can be used as functional ingredient with high protein digestibility and a good source of probiotics. Action of β amylase present in soya on the starch and dextrins of banana might have resulted in the production of more fermentable sugar, maltose and better viability of *L. acidophilus* in the food mixtures. Soya flour when added to the fermenting corn mash, primarily increased and accelerated the production of organic acids through the hydroclastic action of the β amylase enzyme of soya beans on the starch and dextrins of corn (Akinrele *et al*., 1968).

Tomato juice is said to stimulate the growth of *L .acidophilus*, resulting in higher viable counts, shorter generation times and improved sugar utilization with more acid production and lower pH (Babu *et al*., 1992). The stimulation could be attributed to greater availability of simple sugars, mainly glucose and fructose, and minerals (i.e. magnesium and manganese) which are growth promoters for *L. acidophilus* (Ahmed and Mital, 1990). Sindhu and Khetarpaul. (2004) also reported that probiotic fermentation of indigenous food mixtures containing tomato pulp using *L.casei* and *L.plantarum* had good acceptability and more viable counts. Harbinder *et al.* (2009) also developed an acceptable and good quality mango soy fortified probiotic yoghurt (MSFPY).

5.7. Storage studies of the selected food mixtures.

In the present study, the best six food mixtures selected were packed in metallised polyester/ polyethylene laminated pouches and kept for shelf life studies for a period of six months, under ambient conditions.

In modern age, food packaging has become very important because of protection of the product from contamination by macro & micro-organisms and their

filth, prevention from loss or gain of moisture, shielding the product from oxygen and to facilitate handling (Ball, 1960).

The packaging materials used and the conditions, under which the products are stored, are important for the quality of products containing probiotic bacteria. Packing materials can also impact on probiotic stability through variations in oxygen permeability (Shah and Ravula, 2000; Miller *et al*., 2002)

Chemical constituents of fermented and unfermented food mixtures on storage

Moisture content of food mixtures were found to be increasing during storage, but there was no significant variation in the moisture content of FFM and UFFM during storage. The moisture pick up can be expected to increase with the advancement in storage period, especially when the relative humidity is higher around the storage vicinity. This result is in line with the findings of Chellammal (1995) in sweet potato, Liya (2001) in taro flour, Pillai (2001) in breadfruit flour, Lakshmy (2003) in banana flour and Sharon (2003) in breadfruit flour.

The titrable acidity in both the food mixtures increased significantly during storage. In FFM, the initial mean value of 2.509 g lactic acid/100g increased to 3.328 g lactic acid/100g after six months and in UFFM the initial mean value of 1.255 g lactic acid/100g increased to 2.002 g lactic acid /100g after six months of storage. Titrable acidity was significantly high in FFM during all the storage periods. The increase in titrable acidity might be due to the accumulation of lactic acid produced by the lactic acid bacteria.

The protein content in both FFM and UFFM was found to be decreasing on storage. The mean protein content in FFM and UFFM which was 9.290 and 7.210 g/100g, decreased significantly to 7.793 and 6.401 g/100g respectively. The reduction in crude protein content in FFM, as observed on storage, may be attributed to an increase in protein catabolism by the fermenting microorganisms which results in the

escape of ammonia, the byproduct of metabolic deamination. Goldin (1998) suggested that total protease can increase the production of free amino acids. The decrease in protein content may also be due to the browning reaction which is accelerated by the increase in moisture content during storage.

The β carotene content in FFM and UFFM decreased significantly on storage in both FFM and UFFM. The mean β carotene in FFM and UFFM which was 514.25 and 515.80 µg/100g, reduced to 402.97 and 403.63 µg/100g respectively in six months of storage. The loss in the beta carotene content in storage may be due to oxidation (Gloria *et al*., 1993). β carotene absorbs oxygen giving rise to inactive, colourless oxidation products. The moisture content in FFM and UFFM was found to be increasing on storage which might lead to the degradation of β carotene on storage.

A significant decrease in the mean starch content of FFM and UFFM (54.720 and 65.880 g/100g to 48.058 and 60.372 g/100g respectively) was observed during six months of storage. The gradual decrease in the starch content with advancement in storage period may be due to conversion of starch to sugars. In fermented foods with *L. acidophilus*, this starch hydrolysis is further enhanced by the microbial amylase which converts starch into fermentable sugars. This was in line with the findings of Esuoso and Bamiro (1995) and Pillai (2001) who reported a decrease in the starch content in breadfruit flour on storage. Lakshmy (2003) also reported a decrease in the starch content in banana flour on storage.

The mean reducing sugar, total sugar and TSS content in FFM and UFFM increased significantly on storage. The increase in TSS with storage in FFM may be due to the enhanced breakdown of starch and protein into sugars and soluble amino acids as energy source for the viable bacteria. The starch having been formed in the storage cells and tissues may become transformed into sugars particularly sucrose, glucose and fructose. This change is largely dependent upon the conditions of storage such as temperature and time.

The calcium, potassium and iron content in FFM and UFFM decreased significantly during six months of storage. A gradual decrease in the calcium, potassium and iron content of banana flour was observed during storage by Lakshmy (2003).

The thiamine and riboflavin content in FFM and UFFM showed a significant decrease throughout the storage period. The gradual decrease in nutrients in the food mixtures on storage might have also been due to the utilization of nutrients by the microbes growing in the food mixtures. This view has been suggested by Rangaswami and Bagyaraj (2000) who reported that microbes in foodstuffs utilize the nutrients from the food for their needs. IVSD and IVPD of FFM was significantly high in FFM throughout the storage period.

Sensory qualities of the fermented and unfermented food mixtures on storage

The overall acceptability of FFM was more than UFFM throughout the storage period.

The BCGT (Barley flour, milk coprecipitate, sprouted green gram paste and tomato pulp 2:1:1:1 w/w) probiotic food mixture stored for one month was found to be acceptable and the mean score for overall acceptability ranged from 7.0 to 7.1 (Sindhu and Khetarpaul, 2004). Similarly another probiotic food RSMT mixture (Rice, defatted soya flour, skim milk powder and tomato pulp) developed by Rani and Khetarpaul (1999) was also found to be highly acceptable after one month of storage. Angelov *et al.* (2006) developed an oat based probiotic drink and found that there was no change in its sensory qualities after 21 days of refrigerated storage. Heller (2001) observed that fruit juices fortified with probiotic organism *L.rhamnosus* GG exhibited good shelf life and acceptable sensory properties after 7 days of storage.

Total microbial count in fermented and unfermented food mixtures on storage.

An increase in the bacterial and fungal count was observed in both FFM and UFFM on storage.

The total bacterial count in FFM was very high, due to the presence of viable *L. acidophilus* in FFM. Other contaminant bacteria in FFM was very low, may be due to the inhibitory effect of *L. acidophilus* in the food mixtures. Even though there was a reduction in the viable counts due to storage, the bacteriocins produced by *L. acidophilus* might have inhibited the growth of contaminant bacteria in FFM. There was no fungal growth initially in most of the treatments and by the end of six months, the fungal growth ranged between 2.1 to 2.3 which were very low.

The bacterial count in UFFM was more than that in fermented food mixtures. The initial bacterial count in UFFM ranged from 3.0 to 3.4 $x10^7$cfu /g and by the end of six months, the total bacterial count ranged from 8.4 to 9.2 $x10^7$cfu/g. There was a gradual increase in the moisture content of the food mixtures on storage. According to Bera *et al.* (2001), the growth of fungi and bacteria in the food samples are influenced by moisture content, high or low relative humidity, temperature of storage and type of samples. The increase in the bacterial count and fungal count in UFFM can thus be correlated with the increase in moisture content on storage.

According to Bryan (1974), several factors such as quality of raw materials, storage temperature, processing temperature, storage containers, processing technique, the environment in which it is processed, etc. will have an effect on microbial quality of processed foods. The comparatively low count of microflora in UFFM might have been due to low moisture content observed in these food mixtures and also due to the other factors mentioned by Bryan (1974) that affect the total microflora.

Viability of *L .acidophilus* in the selected fermented food mixtures on storage

The viability and stability of probiotics has been both a marketing and technological challenge for industrial procedures. Probiotic foods should contain specific probiotic strains and maintain a suitable level of viable cells during the products shelf life. Before probiotic strains can be delivered to the consumers, they must first be able to be manufactured under industrial conditions and then survive and retain their functionality during storage, and also in the food products into which they are finally formulated. Additionally, they must be able to be incorporated into foods without producing off flavours or textures- they should be viable but not growing. The packaging materials used and the conditions under which the products are stored are also important for the quality of the products.

The viability of *L.acidophilus* in the present study was found to be decreasing significantly on storage. Initially the viable counts varied from 137 to 282 $x10^7$cfu/g and after six months, the viable count varied from 70 to 133 $x10^7$cfu/g. Eventhough there was a reduction in viable count, the viable count after six months of storage (8.85 to 9.12 log cfu/g) was within the desired level of probiotic organism as recommended by Shah *et al*. (1995)

One of the requirements for microorganisms to be used as dietary adjuncts is the need to retain viability and activity in the food vehicle before consumption. The main factors for the loss of viability of probiotic organisms have been attributed to the decrease in pH of the medium and accumulation of organic acids as a result of growth and fermentation (Hood and Zottola, 1998; Shah and Jelen, 1990)

A similar study conducted by Haynes and Playne (2002) showed that low fat ice cream was a good vehicle for delivering viable probiotics *L. acidophilus*, *L. paracasei* and *Bifidobacterium lactis* and all maintained viable numbers above 10^6 cfu /g in ice cream over a 12 month shelf life.

Yoon *et al*. (2004) also observed that the viable cell count of *L .acidophilus* and *L. delbrueckii* in the lactic acid fermented tomato juice did not decrease during cold storage and remained at 1.4 x 10^8 and 8.1 x 10^8 /ml respectively after 4 weeks of cold storage.

Wang *et al*. (2007) developed a probiotic peanut flour fermented with 4 strains of lactobacillus including *L.acidophilus* and observed a cell population of 9.48 log cfu/g after 72 h fermentation at 37^{o}C. After 28 days of storage no marked change in the viable count of this strain was observed.

Similar results were reported by Teixeira *et al*. (1995) where higher number of viable cells were obtained by spray drying the food mixture with *L. bulgaricus* in their stationary phase. Prasad *et al*. (2002) reported that storage stability of *L.rhamnosus* HN001 which was heat shocked after the stationary phase has retained their viability after storage. This suggests that, the stationary phase induces various physiological states within the cells similar to starvation conditions and glucose depletion that trigger, multiples stress responses to allow survival of the cell population. Stable probiotic containing baby food formulations and confectionaries have been developed and are currently on the market (Langhendries *et al*., 1995 and Fukushima *et al*., 1997).

A common principle is that the higher the initial cell concentration, the longer the shelf life of the products (Costa *et al*., 2002). In the present study, the initial viable count of *L.acidophilus* was very high in the food mixtures so that they retained high viable counts even after 6 months of storage. Marshall (1991) has also stated that, the cell count at the end of incubation must be sufficiently high to allow upto 90 per cent mortality of probiotic bacteria during storage and yet still leave their number above the desired minimum of 10^6 cfu/ml viable cells.

5.8. Modifications in the composition of the fermented food mixtures

The therapeutic potential of probiotic bacteria in fermented products is dependent on their survival during manufacture and storage. *L. acidophilus* and *Bifidobacterium* spp have been reported to be beneficial probiotic organisms that provide excellent therapeutic benefits. Inclusion of probiotic bacteria in fermented products enhances their value as better therapeutic functional foods. However, insufficient viability and survival of these bacteria remain a problem in commercial food products. By selecting better functional strains and adopting improved methods to enhance their survival including the use of appropriate prebiotcs and the optimal combination of probiotics and prebiotics (synbiotics), an increased delivery of viable bacteria in fermented products to the consumers can be achieved.

International Dairy Federation (IDF) suggests that a minimum of 10^7 probiotic bacteria cells should be alive at the time of consumption per gram of product (Ouwehand and Salminen, 1998). In order for these bacteria to exert positive health effects, they have to reach their site of action alive and establish themselves in certain numbers (Sultana *et al.*, 2000). However, studies indicate that probiotics may not survive in high required numbers when incorporated into dairy products (Kailasapathy and Rybka, 1997). Many studies have also focused on the survival of these bacteria in dairy products under different production and storage conditions (Beal *et al.*, 2001).

The method of increasing probiotic survival depends to the type of food products. Selection of resistant probiotic strains to tolerate production, storage and gastrointestinal tract condition, is one of the important methods. Another way is to adjust the condition of production and storage, for more survival rates. The physical protection of probiotics by microencapsulation is a new method to increase the survival of probiotics (Wojtas *et al.*, 2007). The addition of growth promoting factors or prebiotics, such as starch and oligosaccharides (Crittenden *et al.*, 2001), buffering of yoghurt mixes with whey proteins (Ravula and Shah, 1998) and modulating packaging conditions have improved the survival of bacteria (Miller *et al.*, 2002).

To improve the suitability of the food as a substrate for probiotic, energy sources (e.g. glucose), growth factors (e.g. yeast extract and protein hydrolysates) or suitable antioxidants, minerals or vitamins can be added into it (Kurmann, 1988; Ishibashi and Shimamura, 1993; Dave and Shah, 1998; Gomes *et al*, 1998). The attributes of companion starter cultures, prebiotics, oxygen scavengers, water activity and sugar concentration dramatically influence probiotic survival during product storage (Bruno *et al*., 2002)

Fermented food mixture with sucrose (T3S)

As indicated by Gomes *et al.* (1998), to improve the suitability of the food as a substrate for the probiotic, energy sources (sucrose, glucose etc), and growth factors (protein hydrolysates), minerals or vitamins can be added into it.

The results of the current study revealed that addition of sucrose at 5, 10 and 15 per cent level in T_1, T_3 and T_8 showed a viable count more than their corresponding fermented controls (FC). Maximum viable count (351 $x10^7$cfu/g) was at 5 per cent sucrose level (T_3S).

Storage studies with sucrose added fermented food mixture showed a viable cell count of 208 $x10^7$cfu /g (9.32 log cfu/g) after six months of storage which is more than that observed in its fermented control T3 (133 $x10^7$cfu/g) after storage.

In a similar study by Homayouni *et al.* (2008) *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum* and *Bifidobacterium longum* showed different responses in 10, 15, 20 and 25 per cent sucrose concentrations. *Lactobacillus casei* (Lc01) showed the highest growth rate in all sucrose concentrations. The number of viable *Lactobacillus casei* cells was 110^{10} (cfu mL^{-1}) and *Lactobacillus* genera resist better than *Biffidobacteria* in different sucrose concentrations. The latter was in contrast with the results of Hekmat and McMahon (1992).

Basyigit *et al*. (2006) also reported that the number of LAB in ice cream samples made by fermentation was high in samples containing sucrose and aspartame.

Angelov *et al*. (2006) observed an increase in the cell count in the oat based probiotic drink on the addition of sucrose in concentration of 1.0, 1.5 and 2.0 per cent and the highest viable count of 2.81 log orders for 2.0 per cent sucrose. He suggested the addition of 1.5 per cent sucrose sufficient for obtaining the probiotic product.

The survival of probiotic strains used in a product may be affected by osmotic pressure associated with sucrose (Ziemer and Gibson, 1998; Medici *et al.*, 2004). It was seen that reducing sugars such as maltose can increase the growth rate of *L. acidophilus*, *L. reuteri* (fermentum) and *L. plantarum* (Charalampopoulos *et al.*, 2003). Although sucrose that is not a reducing sugar; it can increase the growth rate of *L. acidophilus* and *L. casei*. Resistance to osmotic pressure of sucrose is a strain dependent factor.

In the present study, the addition of sucrose showed no significant difference in moisture, starch, protein, β carotene, calcium, potassium, iron, IVSD and IVPD when compared to the FC throughout the storage period. A significant increase in the titrable acidity, TSS, reducing and total sugars, thiamine and riboflavin was found in T_3S than FC throughout the storage period.

The initial mean score of 8.24 for overall acceptability of T_3S was reduced to a mean score of 7.886 after six months, against an initial mean score of 7.966 which reduced to 7.588 after six months in fermented control (T_3). The overall acceptability was higher in T_3S than T_3 throughout the storage period which was mainly contributed by the enhanced taste due to addition of sucrose.

Taste of the fermented food mixtures can be improved by the addition of sucrose along with an enhanced viable cell count. Hence addition of sucrose at 5 per cent level can be recommended in the formulation of fermented food mixtures.

Fermented food mixture with sorbitol (T3SB)

The addition of sorbitol at 5, 10 and 15 per cent level in T_1, T_3 and T_8 did not show much change in the viable count of *L.acidophilus* from their respective fermented controls (FC). Maximum viable count (284 $x10^7$cfu/g) was at 5 per cent level in T_3SB. Storage studies with sorbitol added fermented food mixture showed a viable cell count of 134 $x10^7$ cfu/g (9.14 log cfu/g) after six months of storage, which was not much different from (T_3) fermented control (133 $x10^7$cfu/g) after storage.

Angelov *et al*. (2006) also found that the addition of sweeteners like aspartame, sodium cyclamate, saccharain and huxol had no effect on the dynamics of the fermentation process and on the viability of the starter culture, *Lactobacillus plantarum* B28 during the storage of oat based probiotic drink.

In the present study, sorbitol added fermented food mixtures did not show a reduction in the viable count from that of the fermented control throughout the storage period. But there was a reduction in the viable cell count due to storage which reached to a viable count of 134 $x10^7$cfu/g (9.14 log cfu/g) after six months.

The effects of sorbitol and monosodium glutamate upon survival during storage of freeze-dried *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Enterococcus durans* and *Enterococcus faecalis* were also examined by Carvalho *et al*. (2000) and found no significant differences in survival during freeze-drying after addition of sorbitol or monosodium glutamate .However, these compounds were found to increase the stability of most strains during long-term storage.

A good protectant should provide cryoprotection of cells during freeze drying, be easily dried, and provide a good matrix to allow stability and ease of rehydration (Costa *et al*., 2002). Sorbitol and monosodium glutamate (MSG) have been reported as efficient protectants (Linders *et al*. 1997). An increase and residual activity and viability during drying following the addition of sorbitol to the drying medium, has been

previously reported for various organisms (Abadias *et al*., 2001). Although the mechanism of protecting living cells by polyols is not fully understood, three hypotheses have been proposed so far that may be involved in cell protection (Wisselink *et al.*, 2002): (i) maintenance of turgor, resulting from the accumulation of sorbitol at low water activity (Kets *et al*., 1996); (ii) stabilisation of the structures of membrane lipids and proteins at low water activity (Yoo and Lee, 1993); and (iii) prevention of oxidative damage by scavenging of free reactive oxygen radicals (Leslie *et al.,* 1995).

Sorbitol has a strong protective effect upon the survival of many strains of lactobacillus during storage. Similar results were also obtained by Fonseca *et al.* (2000), who have shown that there is no effect of glycerol during freezing of *Streptococcus thermophilus*, although a positive effect was observed during frozen storage. Substantial increases in residual activity and viability after drying, following addition of sorbitol to the drying medium, have been reported for various organisms (De Valdéz *et al.,* 1983).

In this study addition of sorbitol did not show any significant difference in the chemical constituents compared to FC throughout the storage period.

The overall acceptability of T_3SB showed an initial score of 8.126, which reduced to a mean score of 7.78 after storage, whereas in the fermented control (T_3) this was 7.966 and 7.588 respectively. Thus there was a considerable enhancement in the overall acceptability when sorbitol was added at 5 per cent level in T_3.

Hence sorbitol, which is widely used as a low calorie sweetner can be recommended for incorporation in the fermented food mixture to improve the acceptability of the product especially taste, without any change in the viability of the cells from that of its fermented control (T_3).

Fermented food mixture with wheat bran (T_3W)

Dietary carbohydrate that are indigestible or escape complete digestion in the small intestine may provide carbon and energy source for the bacteria residing within the large bowel. Hence these dietary components are termed prebiotics and are produced commercially as functional food ingredients for improving intestinal health. Many of the proposed health benefits are attributed to dietary fibres.

The beneficial role of dietary fibre in human nutrition has lead to a growing demand for incorporation of novel fibres into foods (Ramaswamy and Basak, 1992). Some dietary fibre like polysaccharides has shown promise as prebiotics (Brown *et al*., 1997). Prebiotics might influence the growth and survival of the probiotics by influencing the growth and metabolites of both the probiotics and the starter.

As per the results, of the present study the addition of wheat bran at 5, 10 and 15 per cent level in T_1, T_3 and T_8 showed a viable count more than their corresponding fermentable controls (FC). Maximum viable count (333 $x10^7$cfu/g) was at 5 per cent level in T_3W. Storage studies with wheat bran added fermented food mixture showed a viable cell count of 189 $x10^7$cfu/g. (9.27 log cfu/g) after six months of storage as against 133 $x10^7$cfu /g in fermented control (T_3) after storage.

In a similar study with fibre incorporated yoghurts by Garcia and McGregor (1997) showed that fibre addition caused an acceleration in the acidification rate of the experimental group yogurts, and most of the fortified yogurts also showed increased viscosity.

Citrus fibres enhanced *L. acidophilus* CECT 903, and *L. casei* CECT 475 survival in MRS during refrigerated storage and decreased with storage time **(**Sendra *et al.,* 2008)

Addition of wheat bran showed a significant increase in the titrable acidity and fibre content in T_3W than FC. There was no significant difference in all the other chemical constituents compared to FC throughout the storage period.

The overall acceptability was higher in T_3W which showed an initial mean score of 8.144 and 7.774 after six months storage, whereas this was 7.962 and 7.584 in fermented control (T_3).

Fermented food mixture with skimmed milk powder (T_3SK)

As per the results of the present study, the addition of skimmed milk powder at 5, 10 and 15 per cent level in T_1, T_3 and T_8 showed a viable count more than their corresponding fermentable controls (FC). Maximum viable count (347 $x10^7$cfu/g) was at 5 per cent level in T_3SK. Storage studies with skimmed milk powder added fermented food mixture showed a viable cell count of 194 $x10^7$cfu/g (9.30 log cfu/g) after six months of storage which is much higher than that observed in its fermented control (T_3) after storage (133 $x10^7$cfu/g).

Skimmed milk powder has been selected as drying medium because it creates a porous structure in the freeze-dried product that makes rehydration easier; it is also believed that proteins in milk provide a protective coating for the cells (Abadias *et al.,* 2001). Supplementing skimmed milk with protective agents may enhance the intrinsic effect of protection during storage to different degrees, depending on the compound added. The ability of a compound to preserve the viability of cells during periods of desiccation has been associated either with the presence of an amino group, a secondary alcohol group, or both (de Valdéz *et al*., 1983).

As indicated by Pascual *et al*., (1999) in the freeze-drying method, glycerol and skimmed milk act as cryoprotective agents to preserve probiotic strain.

Kos *et al*. (2008) also found that skimmed milk acted as lyoprotectant, and improved the viability of *L.acidophilus* M92, *L. plantarum* L4 and *E. faecium* L3 during fermentation and storage.

The addition of skimmed milk powder showed no significant difference in moisture, starch, β carotene, IVSD and IVPD when compared to the FC throughout the storage period. A significant increase in the protein, titrable acidity, TSS, reducing and total sugars, calcium, potassium, iron, thiamine and riboflavin was found in T_3SK than FC throughout the storage period. Overall acceptability was higher in T_3SK, the mean score varying from the initial value of 8.236 which reduced to 7.888 after six months and this was 7.962 and 7.588 respectively in the fermented control (FC).

Probiotics especially *L. acidophilus* with well documented health benefits are traditionally included in dairy products, due to the historical association of lactic acid bacteria with fermented milk. These products require refrigerated transport and storage, this limits the application of probiotics. The inclusion of *L. acidophilus* in a dry food system would therefore have advantages.

Application of probiotic culture in non dairy products and environment represents a great challenge. Viability and probiotic activity must be maintained throughout processing, handling and storage of the product containing the probiotic and has to be verified at the end of shelf life. In the present study, three foods mixtures (T_1, T_3 and T_8) were identified as products with good viability and acceptability even after six months of storage at room temperature. These are products without any food additives having good shelf life. Addition of sucrose (T_3S), wheat bran (T_3W) and skimmed milk powder (T_3SK) at 5 per cent level further enhanced the viable cell count as well as the acceptability of the products. Fermented food mixture with sorbitol was also found to be good without any reduction in the viable count from that of the control (T_3). So, Sorbitol at 5 per cent level (T_3SB) can also be recommended as an acceptable low calorie probiotic food. All these food mixtures contained a viable count ranging from 9.12 and 9.32 log cfu/g after six months of storage under ambient conditions.

Hence, the present study revealed that dry fermented food mixtures T_1 (70 per cent Banana flour + 20 per cent defatted soya flour + 10 per cent mango pulp), T_3 (60 per cent banana flour + 20 per cent defatted soya flour + 20 per cent tomato pulp), T_8 (60 per cent banana flour + 20 per cent defatted soya flour + 10 per cent mango pulp + 10 per cent tomato pulp) along with T_3S (T3 with 5 per cent sucrose), T_3SB (T_3 with 5 per cent sorbitol), T_3W (T_3 with 5 percent wheat bran), T_3SK (T_3 with 5 per cent skimmed milk powder) provide an equally efficient means of administrating the acceptable level of probiotics as dairy products and may therefore extend the use and application of probiotics. The results indicate that further studies with this type of probiotic containing indigenous dry food mixtures are warranted.

5.9. Quantity recommended

The viable count of *L. acidophilus* in the developed probiotic food mixtures at the expiry period (after six months of storage) ranged between 95 to 210 x 10^7 cfu/g and in five grams the viability ranged between 475 to 1040 x 10^7 cfu. This was within the recommended level of the probiotic organism as suggested by Kurmann and Rasic (1991), to assure health benefits. Since the fermented food mixtures were slightly acidic in taste, it can be used with acidic foods like buttermilk, fruit juices etc to enhance their acceptability.

5.10. Cost of the developed food mixtures

The cost of the developed food mixtures ranged between Rs 530 to Rs 550 per 400g. There is no such similar probiotic product available in the market for comparison. In the present study, for the preparation of the food mixtures all the raw materials in limited quantity was purchased from the local market whereas for commercial production, raw materials can be procured in bulk which substantially reduces the cost of the product. For the preparation of the food mixtures at laboratory levels, freeze drying was done in tubes for obtaining small quantity of the mixtures. In commercial production, other sophisticated methods of freeze drying in bulk can be adopted to reduce the cost of the food mixtures.

Future line of work

Modern consumers are increasingly interested in their personal health and expect the food that they eat to be healthy or even capable of preventing illness. Hence, to assure health claims of the developed probiotic food mixtures, clinical studies have to be conducted. More corroborative studies are required to associate changes in gut bacterial populations with physiological aspects in humans. There is good evidence that *L. acidophilus* are safe for human use and able to confer some health benefits on the host, but such benefits cannot be extrapolated without experimentation. Only after conducting such clinical trials these food mixtures can be recommended for specific diseases with well documented therapeutic effects.

Summary

6. SUMMARY

The study entitled "Standardisation and quality evaluation of banana based probiotic fermented food mixtures" was undertaken with the objective to standardise indigenous food mixtures based on banana flour with probiotic fermentation involving *Lactobacillus acidophilus* and to evaluate the nutritional factors, organoleptic qualities and storage stability of food mixtures. Probiotic characteristics like acid and bile tolerance and antimicrobial activity of *L.acidophilus* MTCC 447 was studied and found that the selected strain could survive in a pH range of 2.0 -9.0 and at 3 per cent bile salt concentration . *In vitro* studies with the strain also revealed an antagonistic activity against enteropathogens viz *Salmonella enteritidis, E.coli, Bacillus cereus* and *Staphylococcus aureus. Salmonella enteritidis* was the maximum inhibited pathogen by *L. acidophilus* at pH 3.0.

The foods selected for developing the probiotically fermented food mixtures were banana (Nendran) flour, defatted soya flour, green gram flour, ripe mango, papaya and tomato.From the 56 combinations tried, 14 fermented food mixtures with *L.acidophilus* MTCC 447 were selected statistically by applying Kendall's coefficient of concordance based on their organoleptic qualities.

All the selected 14 food mixtures contained 60-70 per cent banana flour, 20 per cent defatted soy flour/ green gram flour and 10-20 per cent fruit pulps. The variables for *L.acidophilus* fermentation were optimised. For all the treatments, fermentation with 25g of the food mixture at pH 4.5, inoculated with 300µl and incubated at 37°C for 24 h gave the maximum total viable counts of *L.acidophilus* ranging from 9.13 to 9.45 log cfu/g. All the fermented foods along with unfermented controls was freeze dried. Constituents like titrable acidity (2.59 g lactic acid / 100g), protein (7.82g/100g), iron (6.48mg/100g), thiamine (0.073 mg/100g) and riboflavin (0.535 mg/100g) were significantly high in fermented food mixtures. *In vitro* digestibility of starch (82.109 per cent) and protein (85.85 per cent) were also significantly high in fermented food mixtures. Total viable count of *L. acidophilus* ranged from 9.13 to 9.45 log cfu/g. Mean

score of overall acceptability of fermented products varied between 7.9-8.0 in a 9 point hedonic scale by a panel of 10 semi trained judges, where as that of controls were between 7.2 -7.3. The high acceptability for the fermented food mixtures were mainly contributed by the high scores for flavour and taste.

From the 14 fermented food mixtures, 6 fermented food mixtures were statistically selected considering all the quality criteria by computing geometric mean scores. The selected food mixtures T_1, T_2, T_3, T_7, T_8 and T_9 along with their respective controls were packed in metallised poly ester/poly ethylene laminate pouches and kept for storage studies under ambient conditions for a period of six months. Each month quality evaluation was carried out.

After six months of storage maximum titrable acidity (3.377 g lactic acid/100g) was in T_7, starch (48.357 g/100g) in T_2, β carotene (478.23 g/100g) in T_1, protein (8.243 g/100g) in T_3, TSS (11.80°brix) in T_2, reducing sugar (3.17 g/100g) and total sugar (15.78 g/100g) in T_1. Calcium (60.96 mg/100g), potassium (60.53 mg/100g) and iron (377.82 mg/100g) was maximum in T_3. Maximum thiamine (0.023 mg/100g) and riboflavin (0.013 mg/100g) was in T_3 and T_2 respectively. IVSD (85.46 per cent) and IVPD (87.59 per cent) was in T_9 and T_3 respectively. The total bacterial load decreased on storage and fungal count increased with storage. After six months of storage, viable count of *L. acidophilus* in the food mixtures significantly reduced which varied from 8.84 (T_7) to 9.12 (T_3) log cfu/g. Eventhough, the viable count was within the desired level. The overall acceptability of the food mixtures were between 7.42 to 7.59 after six months of storage.

From the six fermented food mixtures with maximum shelf life qualities three fermented food mixtures were statistically selected by applying geometric mean scores. The treatments with maximum geometric mean score were T_1 (70 per cent banana flour, 20 per cent defatted soy flour, 10 per cent mango), T_3 (60 per cent banana flour, 20 per cent defatted soy flour, and 10 per cent tomato pulp) and T_8 (60 per cent banana flour, 20 per cent defatted soy flour, 10 per cent mango and 10 per cent tomato pulp)

Substrate composition was modified by adding sucrose, sorbitol, wheat bran and skimmed milk powder to T_1, T_3 and T_8. The level of these ingredients were standardized as 5 per cent (from trials with 5, 10 and 15 percent) with maximum viable counts of *L.acidophilus* in food mixture T_3.Viable counts in T_3 with 5 per cent sucrose (T_3S) 351 cfu/g x 10^7, with 5 per cent sorbitol (T_3SB) was 284 x 10^7cfu/g, with 5 per cent wheat bran (T_3W) was 333 x 10^7cfu /g and with 5 per cent skimmed milk powder (T_3SK) was 347 x 10^7cfu /g. Thus four treatments (T_3 + sucrose 5 per cent, T_3 + 5per cent sorbitol, T_3 + 5per cent wheat bran and T_3 + 5 per cent skimmed milk powder were subjected to shelf life studies and quality evaluation.

Constituents like TSS, reducing sugars, total sugars were significantly high in T_3S (sucrose added T_3) where as constituents like moisture, starch, protein, β carotene, calcium, potassium, iron, IVSD and IVPD showed no significant difference from that of T_3 after six months of storage. Viable count and overall acceptability showed an increase when compared with their fermented control (T_3). There was no significant difference in any of the nutrients in sorbitol added fermented food mixture compared with the control sample. In wheat bran added food mixture there was a significant increase in the titrable acidity and fiber content. There was no significant difference in moisture, β carotene, starch, IVSD and IVPD in skim milk powder added food mixture. A significant increase was observed in titrable acidity, TSS, reducing and total sugar, protein, calcium, potassium, iron, thiamine and riboflavin in skim milk powder added food mixture when compared with their fermented control.

After modifying the substrate, food mixture T_3S (with added sucrose at 5 per cent level) showed high acceptability and an increase in the viable count of *L.acidophilus* after storage (208 x 10^7cfu/g) when compared to T_3. T_3SB (with added sorbitol at 5 per cent level) showed better acceptability from that of T3 in any aspects and was comparable to T_3 with a viable count of 134 x 10^7 cfu/g after storage. T_3W was also comparable to T_3 but an increase in total viable count (189 x 10^7cfu/g. T_3SK showed an increase in all the nutrients, acceptability and viable count (194 x 10^7 cfu/g.

The present study revealed that dry food mixtures T_1 (70 per cent banana flour, 20 per cent defatted soy flour and 10 per cent mango), T_3 (60 per cent banana flour, 20 per cent defatted soy flour and 10 per cent tomato pulp) and T_8 (60 per cent banana flour, 20 per cent defatted soy flour,10 per cent mango and 10 per cent tomato pulp), T_3S (with 5 per cent sucrose), T_3SB (with 5 per cent sorbitol), T_3W (with 5 per cent wheat bran) and T_3SK (with 5 per cent skimmed milk powder) are fermented food mixtures with good acceptability and desirable viable counts after six months of storage under room temperature. T_3SB (with 5 per cent sorbitol) is also an equally good low calorie probiotic food. All these food mixtures contained a viable count ranging from 9.13 to 9.32 log cfu/g.

The viable count of *L. acidophilus* in the developed probiotic food mixtures at the expiry period (after six months of storage) ranged between 95 to 210 x 10^7 cfu/g and in five grams the viability ranged between 475 to 1040 x 10^7 cfu. This was within the recommended level of the probiotic organism to assure health benefits. Since the fermented food mixtures were slightly acidic in taste, it can be used with acidic foods like buttermilk, fruit juices etc to enhance their acceptability. The cost of the developed food mixtures ranged between Rs 530 to Rs 550 for 400 grams.

It was concluded that it is possible to produce probiotic foods by different processes containing high levels of *L.acidophilus* throughout the storage time in combination with acceptable organoleptic properties. Storage at room temperature, which is common for many types of non- dairy products such as cereal and pulse products, drinks, confectionary etc can create an overwhelming challenge for probiotic stability.

The viability of probiotics has been both a marketing and technological concern for many industrial produces. New processes and formulation technologies will enable both expansion of the range of products into which probiotics can be applied and the use of efficacious strains that currently cannot be manufactured or stored with existing technologies. But there is a need for *in vitro* and *in vivo* tests to better predict the ability

of probiotic organism to function in humans such scientific validation of functional foods will help to satisfy consumer's demand in determining the efficacy for improved health through functional foods.

References

REFERENCES

A.O.A.C. 1970. *Official and Tentative Methods of Analysis.* Eleventh edition. Association of Official Analytical Chemists, Washington, D.C, 1128 p.

A.O.A.C. 1980. *Official and Tentative Methods of Analysis.* Thirteenth edition. Association of Official Analytical Chemists, Washington, D.C, 1018 p.

Abadias, M., Benabarre, A., Teixido, N., Usall, J. and Vinas, I. 2001. Effect of freeze-drying and protectants on viability of the biocontrol yeast *Candida sake*. *Int. J. Fd Microbiol.* 65: 173-182.

Abee, T. 1995. Pore-forming bacteriocins of gram-positive bacteria and self-protection mechanisms of producer organisms. *Microbiol. Lett.* 129: 1–10.

Adams, M. R. and Moss, M.O. 2008. *Food Microbiology*. RSC Publishing, Cambridge, 369p.

Adejuyitan, J. A., Otunola, E. T., Akande, E. A., Bolarinwa, I. F. and Oladokun, F. M. 2009. Some physicochemical properties of flour obtained from fermentation of tigernut (Cyperus esculentus) sourced from a market in Ogbomoso, Nigeria**.** *Afr. J. Fd Sci.* 3(2): 51-55.

Aegerter, P. and Dunlap, C. 1980. Culture of five commonly used acid-producing bacteria on banana pulp. *Appl. Environ. Microbiol*. 39: 937-942.

Agarwal, G.P and Hasija, S.K. 1986. *Microorganisms in the Laboratory*. Print House India Ltd., Lucknow, 155p.

Agte, V.V., Gokhale, M.K. and Chiplonkar, S.A.1997. Effect of natural fermentation on in vitro zinc bioavailability in cereal legume mixtures. *Int. J. Fd Sci. Technol*. 1: 29-32.

Aguirre, M. and Collins, M.D. 1993. Lactic acid bacteria and human clinical infection. *J. Appl. Bact.* 75: 95-107.

Ahmed, B. and Mital, B.K. 1990. Effect of magnesium and manganese ions on the growth of *Lactobacillus acidophilus*. *J. Fd Technol*. 27: 228-229.

Aiba, Y., Suzuki, N., Kabir, A.M.A., Takagi, A. and Koya, Y. 1998. Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotic mucine model. *Am. J. Gastroenterol*. 93: 2097-2101.

Akinrele. A.I., Makanju, A., and Edwards.C.C. 1968. Effect of soya flour on the lactic acid fermentation of milled corn. *Appl. Microbiol.* 17: 186-187.

Alamprese. C., Foschino, R., Rossi, M,, Pompei, C. and Corti, S. 2005. Effects of *Lactobacillus rhamnosus* GG addition in ice cream. *Int. J. Dairy Technol*. 58(4): 200-206.

Alm, I. 1982. Effect of fermentation on B-vitamin content of milk in Sweden. *J. Dairy Sci*. 65: 353-359.

Alm, L. 1983. Survival of salmonella and shigella in fermented milk products with and without added human gastric juice- An in vitro study. *Prog. Fd Nutr. Sci.* 7: 19-23.

Anand, S.K. Srinivasan, R.A. and Rao, L.K. 1984. Antibacterial activity associated with *Bifidobacterium bifidum* I. *Cult. Dairy Prod. J*. 19: 6-8.

Anderson, J.W. and Gilliland, S. E. 1999. Effect of fermented milk (yoghurt) containing *Lactobacillus acidophilus* liansuum cholesterol hyper cholesterolemia in humans. *J. Am. Clin. Nutr*. 18: 43-45.

*Anderson, D. B., McCracken, V. J., Aminov, R. I., Simpson, J. M., Mackie, R. I., Verstegen, M.W.A. and Gaskins, H. R. 2000. Gut microbiology and growth-promoting antibiotics in swine. *Pig News Inf*. 20: 1115–1122.

Angelov, A., Gotcheva, V., Kuncheva, R. and Hristozova, T. 2006. Development of a new oat based probitic drink. *Int. J. Fd Microbiol*. 12: 75-80.

Antonsson, M., Molin, G. and Ardo, Y. 2003. Lactobacillus strains isolated from Danbo cheese and their function as adjunct cultures in a cheese model system. *Int. J. Fd Microbiol*. 85: 159-169.

Antony, U., Sripriya, G. and Chandra. T. S. 1996. Effect of fermentation on the primary nutrients in finger millet *(Eleusine coracana)*. *J. Agric. Fd Chem.* 44 (9): 2616–2618.

Anuradha, S. and Rajeshwari, K. 2005. Probiotic in health and disease. *J. Indian Acad. Clin. Medi.* 6: 67-72.

Anonymous. 2001. Pro- and prebiotics gain popularity in a large US market for gut health. *Nutr. Bus J*. 11: 23-26.

Anonymous. 2007a. World's No. 1 Probiotics drink Yakult launched in India. [on line]. Meri news. Available: http://www.merinews.com. [14 March 2009]

Anonymous. 2007b. Amul's probiotic ice cream gets IDF marketing award [on line]. Diet new. Available: http://www.medindia.net. [04 May 2009].

Anonymous. 2008. *Nestle India: Buy* [on line]. Business Daily from the Hindu group of publications, New Delhi, http://www.thehindubusinessline.com/iw/2008/01/06/stories/2008010651270100.htm [21 June 2009].

Armuzzi, A., Ceemonini, F., Bartolozzi, F., Canducci, F., Candelli, M., Ojetti, V., Cammarota, G., Anti, M., De Lorenzo, A., Pola, P., Gasbarrini, G. and Gasbarrini, A. 2001.The effect of oral administration of *Lactobacillus GG* on antibiotic-associated gastro intestinal side-effects dueins helicobacter pylori eradication therapy. *Aliment. Pharmacol. Ther.* 15(2): 163-169.

*Arora, S., Jood, S., Khetarpaul, N. and Goyal, R. 2008. Effect of germination and fermentation on pH, titrable acidity and chemical composition of pearl millet based food blends. *Acta Alimentaria.* 38: 1107-1115.

Arunachalam, K., Gill, H.S. and Chandra, R.K. 2000. Enhancement of natural immunity function by dietary consumption of *Bifidobacterium lactus* HN019. *Eur. J. Clin. Nutr*. 54:1-4.

Ashenafi, M. and Mehari, T. 1995. Some microbiological and nutritional Ethiopian fermented beverages. *Ethiopian J. Hlth. Dev*. 9: 105-109.

Aso, Y. and Akazan, H. 1992. Prophylactic effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer. *Urology Int*. 49: 125-129.

Babu, R., Latha, S. and Thompkinson, D.K. 2009. Microencapsulation for enhancing the survival of probiotic *Lactobacillus paracasei* S233. *J. Fd Technol*. 46(4): 325-330.

Babu, V. Mital, B.K., and Garg, S.K. 1992. Effect of tomato juice addition on the growth and activity of *Lactobacillus acidophilus*. *Int. J. Fd Microbiol*. 17: 67-70.

Bajaj, J. S., Saeian. K., Christensen, K.M., Hafeezullah, M., Varma , R. R., Franco , J., Pleuss, J. A., Krakower, G., Hoffmann, R. G. and Binion, D.G. 2008. Probiotic

yogurt for the treatment of minimal hepatic encephalopathy. *Am. J. Gastroenterol.* 103(7): 1707-1715.

Ball, C. O. 1960. Effects on color changes in packaging of fresh meat cuts. *Natl. Provisional*. 143 (47): 10-12.

Ballongue, J. 1993. *Lactic acid bacteria*. Marcel Dekkar Inc, New York, 126p.

Basappa, S.C., Somashekar, D., Agarwal, R., Suma, K., and Bharathi, K. 1997. Nutritional composition of fermented ragi (Chhang) by phab and defined starter cultures as compared to unfermented ragi (*Eleusine coracana G*.). *Int. J. Fd Sci. Nutr.* 48: 313-319.

Basyigit, G., Kuleaşan, H. and Karahan, G. A. 2006. Viability of human-derived probiotic lactobacilli in ice cream produced with sucrose and aspartame. J. Ind. Microbiol. Biotechnol. *33:176-800.*

Bazzoli, F., Zagari, R. M. and Fossi, S. 1992. *In vivo Helicobacter pylori* clearance failure with *Lactobacillus acidophilus*. *Gastrorenterology* .102: 38-41.

BCC. 2008. BCC Press Release [on line]. Available: http://www.bccresearch.com. [08 Jan. 2009].

Beal, C., Fonseca, F. and Corrieu, G. 2001. Resistance to freezing and frozen storage of *Streptococcus thermophilus* is related to membrane fatty acid composition. *J. Dairy Sci.* 84: 2347-2356.

Bekkali, N., Bongees, M.E.J., Vanden Berg, M.M., Liem, O. and Bennings, M.A. 2007. The role of probiotics mixture in the treatment of childhood constipution: a pilot study. *Nutri. J.* 6: 17-19.

Bera, S. K., Singh, S. P., Dash, P. and Dash, M. M. 2001. Screening groundnut germplasm for pod yield under residual moisture conditions of paddy fallow. Indian *J. Pl. Genet. Resour*. 14: 337-8.

Berada, N., Lemeland, J. F., Laroch, G., Thouvenot, P., and Piala, M. 1991. *Bifidobacterium* from fermented milks: Survival during gastric transit. *J. Dairy Sci*. 74: 409-413.

Berner, L. and O'Donnel, J. 1998. Functional foods and health claim legislation, an application to dairy foods. *Int. Dairy. J*. 8: 355-362.

Bernfeld, P. 1962. *Comparitive Biochemistry*. Academic Press, New York, 335 p.

Biller, J.A., Kartz, A.J., Florus, A.F., Buie, T.M. and Gorbach, S.L. 1995. Treatment of recurrent *chlostridium difficile* colits with lactobacillus GG. *J. Pediatr. Gastroenterol Nutr*. 21: 224-226.

Birge, S.J., Kautmann, H.T., Cautrecassa, P. and Whedon, G.D. 1967. Osteoposis,intestinal lecture deficiency and diatary calcium intake. *N. Engl. J. Med*. 276: 445-449.

Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D. and Webb, C. 2003. Cereal-based fermented foods and beverages. *Fd Res. Int*. 36: 527-543.

Bolin, Z., Libudzisz, Z., and Moneta, J. 1997. Survivability of *Lactobacillus acidophilus* as probiotic adjunct in low-pH environments. *Pol. J. Fd Nutr. Sci*. 7(3): 71-78.

Borriello, S.P., Hamnes, W.P. and Holzapfelw. 2003. Safety of probiotics that contain *lactobacilli* BIF *biclobacteria. Gin Infes Dis.* 36: 775-80.

Borpuzari, T., Borpuzari, R.N. and Deuri, B. 2007. Probiotic characteristics of *Lactobacillus acidophilus* strains isolated from fermented milk. *J. Fd Sci. Technol.* 44(2): 158-160.

Bourlioux, R. and Pochart, P. 1988. Nutritional and health properties of yogurt. *Wld Rev. Nutr. Diet*. 56**:**217-258.

Boutron, M. 1996. Calcium, phosphorus, vitamin D, dairy products and colorectal carcinogenesis: A French case--control study. *Br. J. Cancer*. 74(1):145-51.

Bouvier, M. 2001. Effects of consumption of a milk fermented by the probiotic B. animalis DN-173 010 on colonic transit time in healthy humans. *Biosci. Microflora*. 20**:** 43–48

Brown, I., Warhurst, M., Arcot, J., Playne, M., Illam, R.J. and Topping, D.L. 1997. Fecal numbers of *bifidobacteria* are higher in pigs fed *Bifidobacterium lingum* with high amylase cornstarch than with a low amylase corn starch. *J. Nutr*. 127(9): 1822-1827.

Bruno, F.A., Lankaputhra, W.E.V. and Shah, N. P. 2002. Growth, viability and activity of *Bifidobacterium spp*.in skim milk containing prebiotics. *J. Fd Sci*. 67(7): 2740-2744.

Bryan, G.W. 1974. Adaptation of an estuarine polychaete to sediments containing high concentrations of heavy metals. In: Vernberg, F.J. and Vernberg, W.B. (eds.), *Pollution and physiology of marine organisms*. Academic press, London, pp. 123-135.

Bu, L.N., Chang, M. H., Ni, Y. H., Chen, H. L. and Cheng, C.C.2007. *Lactobacillus casei rhamnosus* Lcr35 in children with chronic constipation. *Pediatr. Int.* 49:485–490.

Buddington, R. K., Williams, C. H., Chen, S. and Witherly, S. A. 1996. Dietary supplement of neosugar alters the faecal flora and decreases activities of some reductive enzymes in human subjects. *Am. J. Clin. Nutr.* 63: 709–716.

Calcinaro, F., Dionisi, S., Marinaro, M., Candeloro, P., Bonato, V. and Marzotti. S. 2005. Oral probiotic administration induces interleukin-10 production and prevents spontaneous autoimmune diabetes in the non-obese diabetic mouse. *Diabetologia*. 48: 1565-1575.

CAST [Council for Agricultural Science and Technology]. 2007. CAST [on line]. Available: http://www.cast-science.org. [09 July 2009].

*Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., F. Malcata, X. and Gibbs, P. 2000. Protective effect of sorbitol and monosodium glutamate during storage of freeze-dried lactic acid bacteria. *Le Lait.* 83: 203-210.

Chaitow, L., & Trenev, N. 1990. *Probiotics*. Thorsons, London, 213p.

Chapman, H. R. and Sharpe**,** M.E. 1981. Microbiology of cheese. In: Robinson, R. K. (ed.), *Dairy Microbiology*. Applied Science Publishers, London, pp. 157-343.

Charalampopoulos, D., Pandiella, S. S. and Webb, C. 2003. Evaluation of the effect of malt, wheat and barley extracts on the viability of potentially probiotic lactic acid bacteria under acidic conditions. *Int. J. Fd Microbiol*. 82: 133-141.

Chaturvedi, A. 2001. Designer foods for the health conscious consumer. In: Devi, N.L, Kader,V. and Sumathi, S (eds.), *Emerging trends in functional foods*. Centre for

advanced studies in foods and nutrition, post graduate research centre, Achaya NG Agricultural University, Hyderabad, pp. 269-277.

Chavan, U. D., Chavan, J. K. and Kadam, S.S. 1988. Effect of fermentation on soluble protein and *in vitro* protein digestibility of sorghum, green gram and sorghum green blends. *J. Fd Sci*. 53: 1574-1578.

Chellammal, S. 1995. Developing complementary food products based on cassava and sweet potato. Ph.D. thesis, Kerala Agricultural University, Trichur, 253p.

Chopra, S.L. and Kanwar, J.S. 1978. *Analytical Agricultural Chemistry.* Kalyani Publishers, Ludhiana, 210p.

*Christiansen, P. S., Edelsten, D., Kristiansen, J.R. and Nielsen, E.W. 1996. Some properties of ice cream containing *Bifidobacterium bifidum* and *Lactobacillus acidophilus. Milchwissenschaft*. 51:502-504.

Christiansen, P., Nielson, E.W., Vogensen, F.K., Brogren, C.H. and Ardo, Y. 2006. Heat resistance of *Lactobacillus paracasei* isolated from semi-hard cheese made of pasteurised milk. *Int*. *Dairy J*. 16: 1196-1204.

Christiansen, P., Peterson, M.H., Kask, S., Moller, P.L., Peterson, M. and Neilson, E.W. 2005. Anticlostridia activity of *Lactobacillus* isolated from semi-hard cheeses. *Int*. *Dairy J*. 15: 901-909.

Chou, L. and Weimer, B. 1999. Isolation and characterisation of acid and bile tolerant isolates from strains of *L. acidophilus* . *J. Dairy Sci*. 82: 23-31.

Clark, P.A., Cotton, L.N. and Martin, J.H. 1993. Selection of bifido bacteria for use as dietary adjunts in cultured diary foods: ll tolerance to simulated pH of human stomach. *Cult. Dairy prod. J*. 28: 11-14.

Coconnier, M.H., Lievin, V., Bernet-Camard, M.F., Hudault, S. and Serrin, A.L. 1997. Antibacterial effect of the adherin human *lactobacillus acidophilus* strain LB. *Antimicrob. Angents Chemother.* 41: 1046-1052.

Coconnier, M.H., Liévin, V. and Lorrot, M. 2000. Antagonistic activity of *Lactobacillus acidophilus*LB against intracellular *Salmonella enterica* serovar typhimurium infecting human enterocyte-like Caco-2/TC-7 cells. *Appl. Environ. Microbiol.* 64: 4573–4580.

Coconnier, M.H., Kluenhammer, T.R., Keeneis, S., Bernet, M.F. and Serving, A.L. 1992. Protein mediated adhesion of *lactobacillus acidophilus BG2F04* on human enterocyte and mucus secreting cell lines in culture. *Appl. Environ. Microbiol.* 58: 2034-2039.

Collins, J.K., Thormton, G. and O'Sullivan, G.O. 1998. Selection of probiotic strains for human applications. *Int. Dairy J*. 8: 487-490.

Conway , P.L, Gorbach, S.L. and Goldin, B.R. 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *J. Dairy Sci* . 46: 220-222.

Conway, P. L. 1996. Selection criteria for probiotic microorganisms. *Asia Pacific J. Clin. Nutr.* 5: 10–14.

Correia.S., Nunes, A., Duarte, F.I., Barros, A. and Delgadillo, I. 2004. Sorghum fermentation followed by spectroscopic techniques**.** *Fd Chem*. 90: 853-859.

Corzo, G., and Gilliland, S. E. 1999. Measurement of bile salt hydrolase activity from *Lactobacillus acidophilus* based on disappearance of conjugated of bile salts. *J. Dairy Sci.* 82: 466–471.

Costa, E., Usall, J., Teixido, N., Torres, R. and Vinas, I. 2002. Effect of package and storage conditions on viability and efficacy of the of the freeze-dried biocontrol agent *Pantoea agglomerans* strain CPA-2. *J. Stored Products Res*. 38: 329–339.

Crittenden, R., Bird, A.R., Gopal, P., Henriksson, A., Lee, Y.K. and Playne, M.J. 2005. Probiotic research in Australlia, New Zealand and the Asia pacific region. *Curr*. *Pharmaceutical Design*.11: 37-53.

Crittenden, R.G. and Playne, M. J. 1996. Production, properties and applications of food grade oligosaccharides. *Trends Fd Sci*. *Technol*. 7: 353-362.

Crittenden, R.G., Morris, L.F., Harvey, M.L., Tran, L.T., Mitchell, H.L. and Playne, M. J. 2001. Selection of a *Bifidobacterium* strain to complement resistant starch in a synbiotic yoghurt. *J. Appl. Microbiol*. 90: 268-278.

Cunnigham,R. S., Atrne ,S. and Bengmark. S. 2000. Probiotics and immune response. *Am. J. Gastroenterol.* 95: 522-525.

Dariy council of california. 2000. Dairy Council of California home page [on line]. Available:http://www.dairycouncilofca.org/pdfs/probiotics.pdf.[5 Jan. 2009].

Dalev, D., Maria, B., Agnieszka, T., Stefan, Z. and Grzegorz, L. 2006. Sensory quality of new probiotic beverages based on cheese whey and soy preparation. *Polish J.* 15: 56-57.

Das, D. 2007. *Amul to launch pro-biotic ice cream ranges* [on line]. The Hindu Bussiness line, New Delhi . Available: http://www.thehindubusinessline.com. [12 July. 2009].

Dave, R.I. and Shah, N. P. 1997. Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *Int. Dairy J*. 7: 31-41.

Dave, R.I. and Shah, N. P. 1998. Ingredient supplementation effects on viability of probiotic bacteria in yogurt. *J. Dairy Sci*. 81: 2804-2816.

*De Porres, E., de Arriloa, M.C., Garcia, R., and Roltz, C. 1985. Lactic acid fermentaion of banana puree. *Lebensmittel-Wissenschaftund-Technologie*. 18: 379-382.

De Roos, N.M. and Katan, M.B. 2000. Effects of probiotic bacteria on diarrhea, lipid metabolism and carcinogenesis: A review of papers published between 1988 an 1998. *Am. J. Clin. Nutr*. 71: 405-411.

De Valdez, G.F., De Giori, G., De Ruiz, H.A.P. and Oliver, G. 1983. Protective effect of adonitol on lactic acid bacteria subjected to freeze-drying. *Appl. Environ. Microbiol*. 45: 302-304.

Deeth, H.C. and Tomine, A.Y. 1981. Yogurt: nutritive and therapeutic aspects. *J. Fd Prot.* .44: 78-86.

Deguchi, Y. and Morishita, T. 1985. Comparitive studies on synthesis of water soluble vitamins among human species of Bifidobacteria. *Agric. Biol. Chem*. 49(7): 13-19.

Dewit, O., Pochart, P. and Desjeux, J.F. 1988. Breathe hydrogen concentration and plasma glucose, insulin and free fatty acid levels after lactose, milk, fresh or heated yogurt ingestion by healthy young adults with or without lactose malabsorption. *Nutrition*. 4:131–135.

Dimer, C. and Gibson, G.R. 1998. An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. *Int. Dairy J.* 8: 473-479.

Dinakar, P. and Mistry, V.V. 1994. Growth and viability of *Bifidobacterium bifidum* in Cheddar cheese. *J. Dairy Sci*. 77: 2854-2864.

Diplock, A.T., Agget, P.J., Ashwell, M., Bornet, F., Feen, E.B. and Robeefroid, M.B. 1999. Scientific concepts of functional foods in Europe: concersus document. *Br. J. Nutr*. 81: 11-27.

Djouzi, Z. and Andrieux, C. 1997. Compared effects of three oligosaccharides on metabolism of intestinal microflora in rats inoculated with a human faecal flora. *Br*. *J*. *Nutr*. 78: 313–324.

Donnet-Hughes, A., Rochat, F., Serrant, P., Aeshlimann, J.M. and Schiffrin, E.J. 1999. Modulation of nonspecific mechanism of defense by lactic acid bacteria: Effectme dose. *J. Dairy Sci.* 82: 863-869.

Donohue, D.C. and Salminen, S. 1996. Safety of probiotic bacteria. *Asia Pac*. *J*. *Clin*. 5: 25-28.

Douad, C. 2007. Naked juice strips probiotics to functional juice. News headlines industry www.nutriengrients-usa.com [27 Sept. 2007].

Dupont, W.D. and Page, D.L. 1985. Risk factors for breast cancer in women with proliferative breast disease. *N*. *Engl*. *J*. *Med*. 312:146-151.

ElHag, M. E., Abdullahi, H. E. and Nabila, E.Y. 2002. Effect of fermentation and dehulling on starch, totalpolyphenols, phytic acid content and in vitro protein digestibility of pearl millet. *Fd Chem*. 77: 193-196.

Esuoso, K. O. and Bamiro, F.O. 1995. Studies on the baking properties of non-wheat flours. *Int. J. Fd Sci. Nutr*. 46: 267-273.

FAO. 1998. Fermented fruits and vegetables: A global perspective. FAO Agricultural Services Bulletin No. 134. Food and agriculture organisation of the united states, Rome, 106p.

Farnworth, E.R.2001. Probiotics and prebiotics. In: William, R.E.C (ed). *Handbook of Nutraceuticals and Functional foods,* CRC press, NewYork, pp. 407-422.

Felley, C.P., Corthesy-thoulaz, I., Rivero, J.I., Sipponen, P., Kaufmann, M. and Bauerteind, P. 2001. Favourable effect of an acidified milk (LC-I) on *Helicobacter pylori* gastritis in man. *Eur. J. Gastroenterol. Hepatol*. 13: 25-29.

Filippo, C., Di Caro, S. , Covino, M. , Armuzzi, A. , Gabrielli, M., Santarelli, L ., En rico, C. N., Cammarota, G ., Gasbarrini, G. and Gasbarrini, A. 2001. Effect of different probiotic preparations on anti-Helicobacter pylori therapy-related side effects: a parallel group, triple blind, placebo-controlled study. *Am. J. Gastroenterol.* 97: 2744 – 2749.

Floch, M.H. and Moussa, K. 1998. Probitics and Dietary fiber. *J. Clin. Gastroenterol*. 27: 99-100.

Fonseca, F.C., Be'al. and Corrieu, G. 2000. Method to quantify the loss of acidification activity of lactic acid starters during freezing and frozen storage. *J. Dairy Res*. 67: 83-90.

Fujioka, M., Mariko, U., Wu, J., Herman, A., Kazuharu, S., Kazuki, K., Ken, T., Kazuhiko, Y. and Yoshiko, I. 2004. Equol, a metabolite of daidzein, inhibits bone loss in ovariectomized mice. *J. Nutr*. 134: 2623-2627

Fukushima, M. and Nakano, M. 1996. Effects of a mixture of organisms, *Lactobacillus acidophilus* or *Streptococcus faecalis* on cholesterol metabolism in rats fed on a fat and cholesterol enriched diet. *Br. J. Nutr*. 76: 857-867.

Fukushima, Y., Li, S.T., Hara, H. and Terada, A. 1997. Effect of follow up formula containing *Bifidobacteria* (NAN BF) on fecal flora metabolites in healthy children. *Biosci. Microflora*. 16: 65-72.

Fuller, R. 1993. Probiotic foods. Current use and future developments. *Int. Fd Ingred*. 3: 23-26.

Fuller, R. 1992. *Probiotics: The Scientific basis*. Chapman and Hall, London, 234p.

Fuller, R. 1989. Probiotics in man and animals. *J. Appl. Bacteriol.* 66: 365-378.

*Garcia, E. F. and McGregor, J.U. 1997. Fortification of sweetened plain yogurt with insoluble dietary fiber. *Zeitschrift für Lebensmitteluntersuchungund forschung*. 204: 433 - 437

Gardiner, G., Ross, R.P., Collins, J.K., Fitzgerald, G. and Stanton, C. 1998. Development of a probiotic cheddar cheese containing human-derived Lactobacillus paracasei *strains.* Appl. Environ. Microbiol*. 64: 2192–2199.*

Gardiner. G., Stanton, C., Lynch, P. B., Collins, J.K., Fitzgerald, G. and R. P. Ross. 1999. Evaluation of cheddar cheese as a food carrier for delivery of a probiotic strain to the gastrointestinal tract. *J Dairy Sci*. 82 (7): 1380-1389.

Giannella, R.A., Broitman, S.A. and Zamcheck, N. 1972. Gastric acid barrier to ingested microorganisms in man: studies *in vivo* and *in vitro. Gut*. 13: 251-256.

Gibson, G. R., Beatty, E. R., Wang, X. and Cummings, J. H. 1995. Selective stimulation of *bifidobacteria* in the human colon by oligofructose and inulin. *Gastroenterology*. 108: 975–982.

Gibson, G.R. and Roberfroid, M.B. 1995. Dietary modulation of the human colonic microbiotica: introducing the concept of prebiotics. *J Nutr.* 125: 1401-1412.

Gibson, G.R., McCartney, A.L. and Rastall, R.A. 2005. Prebiotics and resistance to gastrointestinal infections. *Br. J. Nutr*. 93: 31-34.

Gill, H.S.1998. Stimulation of Immune system by lactic cultures. *Int Dairy J*. 8: 534-544.

Gill, H.S., Rathefurd, K.J., Prasad, J. and Gopal P.K. 2000. Enhancement of natural and acquired immunity by *Lactobacillus rhamnosus (HN001), Lactobacillus acidophilus (HN017) and Bifidobacterium lactis* (HN019). *Br. J. Nutr*. 83: 167-176.

Gill, H.S., Cross, M.L., Ratherfurd, K.J. and Gopal, P.K. 2001. Dietary probiotic supplementation to enhance cellular immunity in the elderly. *Br. J. Biomed Sci.* 57(2): 94-96.

Gilliland, S. E. and Speck, M. L. 1977. Antagonistic action of *Lactobacillus acidophilus* toward intestinal and food borne pathogens in associative cultures. *J. Fd Prot*. 40: 820–823.

Gilliland, S. E. and Walker, D. K. 1990. Factors to consider when selecting a culture of *L. acidophilus* as a dietary adjunct to produce a hypercholesterolemic effect in humans. *J. Dairy Sci*. 73: 905–909.

Gilliland, S.E. and Kim, H.S.1984. Effect of viable starter culture bacteria in yogurt on lactose utilization in humans. *J. Dairy Sci*. 67: 1- 6.

Gilliland, S.E., Nelson, C.R. and Maxwell, C. 1985. Assimilation of cholesterol by *Lactobacillus acidophilus*. *Appl. Environ. Microbiol*. 49: 377-381.

Gionchetti, P., Rizzello, F., Verturi, A., Brigidi, P., Matteuzzi, D., Bazzocchi, G., Poggioli, G., Miglioli, M., and Campieri, M. 2000. Oral bacteriotheraphy as maintenance treatment in patients with chronic pocichitis: A double blind, placebo-contolled trial. *Gastroenterol*. 119: 305-309.

Gittleman, A.L. 2004. *Probiotics for the 21st Century* [on line]. Natura Doc,US. Available:http://www.naturodoc.com/library/detox/probiotics.htm[10June 2009]

Gloria, M. B. A., Grulke, E.A. and Gray, J. I. 1993. Effect of type of oxidation on beta-carotene loss and volatile products formation in model systems. *Fd Chem.* 46 (4): 401-406.

Goldin, B.R. and Gorbach, S.L. 1984. The effect of milk and lactobacillus feeding on human intestinal bacterial enzyme activity. *Am. J. Clin. Nutr*. 39: 756-761.

Goldin, B.R.1998. Health benefits of probiotics. *Br. J. Nutr* .80: 203-207.

Gomes, A. M. P., Malcata, F.X., Klaver. F.A.M. and Grande, H.G. 1995. Incorporation and survival of *Bifidobacterium* sp. strain Bo and *Lactobacillus acidophilus* strain Ki in a cheese product. *Neth. Milk Dairy. J.* 49:71-95.

Gomes, A. M. P., Malcata, F.X., Klaver. F.A.M. and Grande, H.G. 1998. Growth enhancement of *Bifidobacterium* lactis Bo and *Lactobacillus acidophilus* Ki by milk hydrolyzates. *Neth. Milk Dairy. J.* 81: 2817-2825.

Goncu, A. and Alpkent, Z. 2005. Sensory and chemical properties of white pickled cheese produced using kefir, yoghurt or a commercial cheese culture as a starter. *Int. Dairy J.* 15: 771-776.

Gorbach, S.L., Chavy, T., and Golden, B. 1987. Successful treatment of relapsing clostridium difficile colitris with lactobacillus GG. *Lancet.* 2: 15-19.

Gotoh, T., Yamada, K. and Ito, A. 1998. Chemoprevention of N-nitroso-N-Methyluiea-induced rat mammary cancer by miso and tamoxifen, alone and in combination. *Jpn J. Cancer Res.* 89(5): 487-95.

Goyal, R. and Khetarpaul, N.1994. Effect of fermentation on HCl- extractability of minerals from rice-defatted soy flour blends. *Fd Chem*. 50: 419-422.

Grajek, W., Anna, O. and Anna, S. 2005. Probiotic, prebiotics and antioxidants as functional foods.www.actabp.pl [23 April 2009].

Guandalini, S., Pensabene, L., Zikri, M.A., Dias, J.A., Casali, L.G., Hoekstra, H., Kolacek, S., Massar, K., Micetic-Turk, D., Papadopoulou, A., de Sousa, J.S., Sandhu, B., Szajewska, H. and Weizman, Z. 2000. *Lactobacillus GG* administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. *J. Pediatr. Gastroenterol. Nutr.* 30: 54–60.

Guarner, F. and Malagelada, J.R. 2003. Gut flora in health and disease. *Lancet.* 361: 512-519.

Guarner, F., and Schaafsma, G.J. 1998. Probiotics. *Int. J. Food Microbiol*. 39: 237-238.

Gulewicz, P., Ciesiodea, D., Frias, J., Vidal-Valverde, C., Frejnagel, S., Trojanowska, K. and Gulewicz, K. 2003. Simpke method of isolation and purification of galactosides from legumes. *J. Agric. Fd Chem*. 48: 3120-3123.

Gupta, P., Andrew, H., Kirschner, B.S. and Guandalini, S. 2000. Is *Lactobacillus GG* helpful in chicken with crohn's disease? Results of a preliminary,open latial study. *J. Pediatr. Gastroenterol Nutr*. 31: 453-457.

Gupta, P.K., Mittal, B.K., and Garg, S.K. 1996. Inhibitory effect of *Lactobacillus acidophilus* against different pathogens in milk . *J. Fd Sci. Technol*. 3: 147-149.

Hamad, A. M. and Fields, M. L. 1979. Evaluation of the protein quality and available lysine of germinated and fermented cereal. *J. Fd Sci*. 44: 456- 458.

Hansen, E. B. 2002. Commercial bacterial starter cultures for fermented foods of the future. *Int. J. Fd Microbiol*. 78: 119-131.

Harbinder, K., Mishra , H. N. and Pradyuma, K. 2009. The effect of yoghurt bacteria and probiotic culture on the textural charachteristics of a mango soy fortified probiotic yoghurt (MSFPY). *Int. J. Fd Sci. Technol*. 44(2): 415-424.

*Harry, A.P. and Leo, F. 1920. Studies on the transformation of the inter final flora with special reference to the implantation of *bacillus acidophilus* 2 feeding experiments on man. NAS. 6: 704-705.

Hart, A., Stagg, A. J. and Kamm, M.A. 2003. Use of Probiotics in the Treatment of Inflammatory Bowel Disease. *J. Clin. Gastroenterol*. 36(2): 111-119.

Hata, Y., Yamamoto, M., Ohni, M., Nakajima, K., Nakamara, Y. and Atakano, T. 1996. A placetro-controlled study of the effect of sour milk on blood pressure in hyoertensive subjects. *Am. J. Clin. Nutr*. 64: 767-771.

Haynes, I. N. and Playne, M. J. 2002. Survival of probiotic cultures in low fat ice cream. *Aust. J. Dairy Technol*. 57: 10-14.

HeFang, F., Elina, T., Heikki, A. and Seppo.S. 2000. Modulation of humoral immune response through probiotic intake. *Immunol. Med. Microbiol*. 29: 147 -152.

Hekmat, S. and Mcmahon, D. J.1992. Survival of *Lactobacillus acidophilus* and *Bifidobacteriumbifidum* in ice cream for use as a probiotic food. *J. Dairy Sci*. 75(6): 1413-1422.

Heller, K. J. 2001. Probiotic bacteria in fermented foods: product characteristics and starter organisms. *Am. J. Clin. Nutr*. 73**:** 374–379.

Hemalatha, R. and Rao, S.G.M. 2009. *Healthy bacteria* [on line]. The Hindu, New Delhi.Available:http://www.hindu.com/mag/2009/03/01/stories/2009030150200600.htm [23 May 2009]

Hesseltine, C.W. 1983. The future of fermented foods. *Nutr. Rev*. 41: 293 -301.

Heyman, M. and Menard, S. 2002. Probiotic micro organism: how many affect intertinal pathopysiology cellular and molecular life science. *Cell Mol. Life Sci.* 59: 1-15.

Hilliam, M. 2000. Functional food: How is the market? *Wld. Fd Ingredients*. 12: 50-53.

Hill, M.J., Goddard, P. and Williams, R.E. 1971. Gut bacteria and etiology of cancer of the breast. *Lancet.* 2: 472-473.

Hillier, S.L., Krohn, M.A., Rabe, L.K., Klebanoff, S.J. and Eschenbach, D.A. 1993. The normal vaginal flora, H_2O_2-producing *Lactobacilli,* and bacterial vaginosis in pregnant women. *Clin. Infect. Dis*. 16: 273–281.

Hilton, E., Kolakowski, P., Singfer, C., Smith, M. 1997. Efficency of *Lactobacillus GG* as a diarchoral preventive in traveler. *J. Travel Med*. 4: 41-43.

Hilton, H.D. and Isenberg, P. 1992. Ingestion of yogurt containing *Lactobacillus acidophilus* is prophylaxis for candidal vaginitis. *Ann. Int. Med*. 116: 353-57.

Holcomb, J. E. and Frank, J. F. 1991 Viability of *Lactobacillus* acidophilus and *Bifidobacterium bifidum* in soft-serve frozen yogurt. *Cult. Dairy Prod. J. 26: 4-5.*

Homayouni**,** A., Ehsani**,** M. R., Azizi**,** A., Razavi**,** S. H., and Yarmand, M.S. 2008**.** Growth and survival of some probiotic strains in simulated ice cream conditions. *J. Appl. Sci*. 8: 379-382.

Honma, N. 1988. Bifidobacteria as a resistance factor in human beings. *Bifidobacteria Microflora*. 7: 35-43.

Honma, N. and Ohtani, K. 1987. On effects of lactic acid bacteria. Part II. Clinical effects. *New Med. Clin*. 36(1): 75- 77.

Hood, S. K. and Zottola, M. L. 1998. Effect of low pH on the ability of *Lactobacillus acidophilus* to survive and adhere to human intestinal cell. *J. Fd Sci*. 53: 1514-1516.

Hosoda, M., He, F., Hiramatu, M., Hashimoto, H. and Benno, Y. 1996. Effects of *Lactobacillus GG* strain intake on fecal microflora and defecation in healthy volunteers. *Bifidus*. 8: 21-28.

Huis, J.H.I. and Havenaar, R. 1992. Probiotics : A general view. In: Wood, B.J.B (ed.), *The lactic Acid Bacteria in Health and Disease*. Chapman & Hall, New York, pp 209-224.

Institute of Medicine. 1998. *Standing Committee on the Scientific Evaluation of Dietary Reference Intakes: Proposed Definition and Plan for Review of Dietary Antioxidants and Related Compounds*. National Academy Press, Washington DC, 545p.

Ishibashi, N. and Shimamura, S. 1993. *Biffidobacteria*: Research and development in Japan. *Fd Technol*. 47: 29-34.

Isolauri, E. 2001. Probiotics in prevention and treatment of allergic disease. *Pediatr. Allergy Immunol.* 12(514): 56-59.

Isolauri, E., Joensuu, J. and Suomaainen, H. 1995. Improved immunogenecity of oral DX RRV reassortant rotavirus vaccine by *L.casei* GG vaccine. *Pediatr. Infect. Dis*. *J.* 14: 107-11.

Isolauri, E., Juntunen, M., Rautanen, T., Silleanaukee, P., Koivula, T. and Atiuman. 1991. *Lactobacillus* strain C *Lactobacillus casei* sp strain GG) promotes recovery from acute diarrhoe in children. *Pediatrics.* 88: 90-97.

Isolausi, E., Arvola, T., Sutas, Y., Moilanen. E. and Salmanien, S. 2000. Probiotics in the management of atopic eezema. *Clin. Exp. Allergy*. 30: 1604-10.

Ito, M., Deguchi, Y., Matsumoto, K., Kimura, M., Onodera, N. and Yajima, T. 1993. Influence of galactooligosaccharides on the human fecal microflora. *J. Nutr. Sci. Vitaminol.* 39: 635–640.

Iwana, H. and Masuda, H. 1990. Microbiology of fermented milks. In: Robinson, R. K. (ed.), *Dairy Microbiology*. Elsevier Science Publishers Ltd., London, 280p.

Jackson, M.L. 1973. *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd., New Delhi, 299p.

Jacob, K. 2008. *Mother Dairy launches probiotic milk for kids*. Just foods. Available:http://www.just-food.com/article.aspx?id=101778 [3 May 2009].

James, W., Anderson, M.D. and Stanley, E. 1999. Effect of fermented milk (Yogurt) containing *Lactobacillus acidophillus* L1 on Serum cholesterol hypercholestrolemic human. *J. Am. Coll. Nutr*. 18: 43-50.

Jaspers, D.A., Massey, L.K. and Leudecke, L. 1984. Effect of consuming yogurt prepared with three culture strains on human serum lipoproteins. *J. Fd. Sci*. 49: 1178-1181.

Jellinek, G. 1985. *Sensory Evaluation of Food - Theory and Practice.* Ellis Horwood Ltd. Chichester England and VCH Verlagsgerellschaft mbh, Weinhein, Germany, 218p.

Johnson, M. C., Ray, B., and Bhowmik, T. 1987**.** Selection of *Lactobacillus acidophilus* strains for use in acidophilus products. *Antonie van Leeuwenhoek.* 53: 12-14.

Jood , S. and Khetarpaul, N. 2005. Improving nutritional quality of course cereals through probiotic fermentation. *Processed Fd Ind*. 23: 17-25.

Kabir, A.M., Aiba, Y., Takagi, A., Miwa, T. and Koga, Y. 1997. Prevention of helicobacter pylori infection by *lactobacilli* in a gnotobiotic musine model. *Gut* 41: 49-55.

Kaila, M., Isolauri, E., Soppi, E., Virtanem, E., Laine, S. and Arrilommi, H. 1992. Enhancement of the circulating antibody secreting cell response in human diarrhaoe by a human *Lactobacillus* strain. *Paediatr. Res*. 32: 141-144.

Kailasapathy, K., and Rybka, S. 1997. *Lactobacillus acidophilus* and *Bifidobacterium* spp. and their therapeutic potential and survival in yogurt. *Aust. J. Dairy Technol.* 52: 28–33.

Kajander, K., Hatakka, K., Poussa, T., Farkkilä, M. and Korpela, R. 2005. A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: A controlled 6-month intervention. *Alimentary Pharmacol. Ther*. 22(5): 387-394.

Kalliomaki, M., Salminen, S., Arrvilommmi, H., Kevo, P., Koskilhan, P. and Isolauri, E. 2001. Probiotics in primary prevention of atopic disease: A randommised placetro-controlled trial. *Lancet.* 357: 1076-1079.

Kang, K. H., Shin, H. J., Park, Y. H. and Lee, T. S. 1989. Studies on antibacterial substances by lactic acid bacteria- putrifaction and some properties of antibacterial substances by *Bifidobacterium longum*. *Korean J. Dairy Sci*. 11: 204-206.

Kao, C. and Robinson, R. J. 1978. Nutritional aspect of fermented foods from chickpea, horsebean and soybeans. *Cereal Chem*. 55: 512-514.

Katz, S.E. 2003. *Wild Fermentation: The Flavor, Nutrition, and Craft of Live-Culture Foods*. Chelsea Green Publishing Company, White River Junction, 234p.

Kaufman, D. W., Kelly, J. P., Curhan, G. C., Anderson, T. E., Dretler, S. P., Preminger, G. M. and Cave, D. R. 2008. Oxalobacter formigenes May Reduce the Risk of Calcium Oxalate Kidney Stones. *J. Am. Soc. Nephrphol*. 19: 1197-1203.

Kets, E. P.W., Galinski, E.A., De Wit, M., De Bont, J.A.M. and Heipieper, H. J. 1996. Mannitol, a novel bacterial compatible solute in *Pseudomonas putida S12*. *J. Bacteriol.* 178: 6665-6670.

Khedkar, J. N., Dave, J. M and Sannabhadti, S.S. 1998. Antibacterial activity associated with Bifidobacterium adolescentis. *J. Fd Sci. Technol*. 35(6): 527-529.

Khetarpaul, N. 2005. *Food microbiology*. Daya publication house , Delhi, 469p.

Kim, H.S., Camilleri, S., McKinzie, S., Lempke, M.B, Burton, D.D., Thomforde, R.M. and Zinsmeister, A.R. 2003. A standardized controlled trial of a probiotic VSL 3, a gut gut transit ans symptoms in diarrhea predominant irritable bowel symdrome. *Alimentary Pharmacol. Therapeutics*. 17:895-904.

Kim, H.S. and Gilliland, S.E. 1983.*Lactobacillus acidophilus* as dierary milk adjunct aid lactose digestions in humans. *J. Dairy Sci*. 66: 959-66.

Kirjavainen, P.V., Saminen, S.J. and Isolauri, E. 2003. Probiotic bacteria in the management of atropic disease: undiscovering the importance of viability *JPGN*. 36(2): 223-227.

Klaenhammer , T.R. and Kullen, M. J.1999. Selection and Design of Probiotics. *Int. J. Fd Microbiol.* 50: 45-57.

Klaver, F.A.M. and Meer, R.V.D. 1993.The assumed assimilation of cholesterol by *lactobacilli* and *bifidobacterium bifidum* is due to their bile salt deconjugating activity. *Appl. Environ. Microbiol*. 59: 1120-1124.

Klayraung, I., Viernstein, H., Sirithunyalug, J. and Okonogi, S. 2008. Probiotic properties of *Lactobacilli* isolated from Thai Traditional Food. *Sci. Pharamacol*. 76: 485-503.

Koebnick, C., Wagner, I., Leitzmann, P., Stern, U. and Zunft, H. J. 2003. Probiotic beverage containing Lactobacillus casei Shirota improves gastrointestinal

symptoms in patients with chronic constipation. *Cancer J. Gastroenterol.* 17**:** 655–659.

Kohajdova, Z. and Karovicova, J. 2007. Fermentation of cereals for specific purpose. *J. Fd Nutr. Res*. 46: 51-57.

Kolars, J.C., Levitt, M.D., Aouji, M. and Saviano, D.A .1984. Yogurt -An autodigesting source of lactose. *New Engl. J. Med.* 310:.1-3.

Koop, H.L. 2001. Prophylactic and therapectic uses of probiotics. *J. Am. Dietetic Assoc.* 56: 98-104

Kosikowski, F. 1977. *Cheese and fermented milk foods*. Edwards Brothers Inc., Mich, 260p

Kos, B., Suskovic, J., Beganovic, J., Gjuracic, K., Frece, J., Iannaccone, C. and Canganella, F. 2008. Characterization of the three selected probiotic strains for the application in food industry. *Wld. J. Microbiol. Biotechnol*. 24: 699-707.

Krishnakumar, V. and Gordon, I.R. 2001.Probiotics challenges and opportunities. *Dairy lnd. Int*. 66(2): 38-40.

Kruis, W., Schutz, E., Fric, P., Fixa, B., Judmaier, G. and Stolte, M. 1997 Double – blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining re mission of ultrative colitis. *Aliment. Pharmacol. Ther* .11:853-858

Kruis,W. 2004. Antibiotics and probiotics in inflammatory bowel disease. *Aliment. Pharmacol. Ther*. 20(4): 75 – 78.

Kurmann, J.A. 1988. Starter with selected intestinal bacteria. *Int. Dairy Fed*. 23: 40-41

Kurmann, J.A. and Rasic, J.L. 1991. The Health potential of products containing Bifidobacteria. In: Robinson, R.K. (ed.). Therapeutic properties of fermented milks. Elsevier Applied Science Publishers, Ltd; London, pp. 117- 158.

Labayen, I., Forga, L., Gonza´lez, A., Lenoir, I. and Martinez, A.J. 2001. Relationship between lactose digestion, gastrointestinal transit time and symptoms in lactose malabsorbers after dairy consumption. *Aliment. Pharmacol. Ther.* 15: 543-549.

Lakshmy, P.S. 2003. Evaluation of fruit quality in banan 'Nendran' (*Musa* AAB). M.Sc. (Hort.) thesis, Kerala Agricultural University, Trichur, 71p.

Lambert, J. and Hull, R. 1996. Upper gastro intestinal tract disease and probiotics. *Asia Pacific J. Clin. Nutr.* 5: 31-35.

Lampe, J.W., Gustafson, D.R., Hutchins, A.M., Martini, M.C., Li. C., Wahala, K., Grandit, G.A., Potter, J.D. and Slavin, J.L. 1999. Urinary isoflavonoid and lignan excretion on a western diet, relation to soy, vegetable and fruit intake. *Cancer Epidimiol. Biomarkers Prev.* 8: 699-707.

Langhendries, J. P., Detry, J., Van Hees, J., Lamboray, J. M., Darimont, J., Mozin, M. J., Secretin, M. C. and Senterre, J. 1995. Effect of a fermented infant formula containing viable bifidobacteria on the faecal flora composition and pH of healthy full-term infants. *J. Pediatr. Gastroenterol. Nutr*. 21: 177–181.

Lankaputhra, W.E.V. and Shah, N.P. 1995. Survival of *Lactic acidophilus* and *Bifidobacterium Spp* in the presence of acid and bile salts. *Cult. Dairy Prod. J.* 30: 2-7.

Lankaputra, W.E.V. and Shah, N.P. 1998. Adhesence of probiotic bacteria in human colonic cells. *Biosci Microflora*. 17:105-113.

Laroia, S. and Martin, J. H. 1990. *Bifidobacteria* as possible dietary adjuncts in cultured dairy products: A review. *Cult. Dairy Prod. J.* 25: 18–22.

Lee, Y.K. and Salminen, S. 1995. The coming age of probiotics. *Trends Fd. Sci. Technol*. 6: 241–245.

Leea, S.J., Gun-Young,H., Leea, J. W., Oha,Y., Parka, A, J., Parka, Y., Pyunb ,Y., and Ahn, S.J. 2005. Analysis of kimchi microflora using denaturing gradient gel electrophoresis. *Int. J. Fd Microbiol*. 102: 143-150.

Lerebours, E., N'Djitoyap, N. C., Lavoine, A., Hellot, M. F., Antoine, J.M. and Colin, R.1989. Yogurt and fermented-then-pasteurized milk: effects of short-term and long-term ingestion on lactose absorption and mucosal lactase activity in lactase-deficient subjects. *Am. J. Clin. Nutr.* 49: 823–827.

Leslie, S.B., Israeli, E., Lighthart, B., Crowe, J. H., and Crowe, L. M.1995. Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. *Appl. Environ. Microbiol*. 61: 3592-3597.

Li, S., Francis, A. K., Rocheford, T. and White, W. S. 2007.Retention of provitamin A carotenoids in high beta-carotene maize (*Zea mays*) during traditional African household processing. *J. Agric. Fd Chem*. 55(26): 10744-10750.

Liem, I.T., Steinkraus, K.H. and Cronk, T.C. 1977. Production of vitamin B-12 in tempeh, a fermented soybean food . *Appl. Environ. Microbiol*. 34(6): 773-6.

Lilly, D.M. and Stillwell, R.H.1965. Probiotics: growth probioting factors produced by microorganisms. *Science*. 147: 747-748.

Lin, H. C., Su, B.H., Chen, A .C., Lin, T.W., Tsai, C.H., Yeh, T.F. and Oh.,W. 2005. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics.* 115: 1- 4.

Lin, M.Y., Savaiano, D. and Harlander, S. 1991. Influence of nonfermented dairy products containing bacterial starter cultures on lactose maldigestion in humans. *J. Dairy Sci*. 74: 87–95.

Lin, S.Y. and Chen, C. 2000. Reduction of cholesterol by *lactobacillus acidophilus* in cultural broth. *J. Fd. Drug Analysis*. 8: 97-102.

Lina, C. A., Alegro, A.H.J., Cardarelli, R.H., Chiu, C.M. and Saad, I.M.S. 2007. Potentially probiotic and synbiotic chocolate mousse. *Fd Sci. Technol*. 40(4): 669-675

Linders, L. J. M., de Jong, G. I.W., Meerdin, G. and Van't Riet, K. 1997. Carbohydrates and the dehydration inactivation of *Lactobacillus plantarum*: the role of moisture distribution and water activity. *J. Fd Engng*. 31: 237-250.

Liong, M.T. and Shah, N. P. 2005. Optimization of cholesterol removal by probiotics in the presence of prebiotics by using a response surface method. *Appl. Envrion. Micrbiol.* 71(4): 1745-1753.

Liya, S. 2001. Value addition and evaluation of nutritional quality in taro. M.Sc. (Hort.) thesis, Kerala Agricultural University, Trichur, 103p.

Lynch, C. M., McSweeney, P. L. H., Fox, P.F., Cogan, T.M. and Drinan, F.D. 1996. Manufacture of cheddar cheese with and without adjunct *Lactobacilli* under controlled microbiological conditions. *Int. Dairy J*. 6: 851-867.

Macfarlane, G.T. and Cummings, J.H. 2002. Probiotics, infection and immunity. *Curr. Infect. Dis*.15: 501-506.

Mack, D.R., Michhail, S., Wei, S., Mc Dougall, I. and Hollingsworth, M.1999. Probiotics inhibit enteropathogenic E coli adhesence in vitro by inducing intestinal muccin gene expression. *Am. J. Physiol*. 276: 941-950.

Madsen, K.L., Doyle, J.S., Jewell, L.D., Tavernini, M.M. and Fedorak, R.N. 1999. *Lactobacillus* species prevents colitis in interleukin 10 genedeficientmice. *Gastroenterology*. 116: 1107-1114.

Majamaa, H. and Isolauri, E. 1997. Probiotics: a novel approach in the management of food allergy. *J. Allergy Clin. Immun*. 99:179-185.

Mann, G. V. 1977. A factor in yogurt which lowers cholesterol in man. *Atherosclerosis*. 26: 335-337.

Mann, G. V. and Spoerry, A. 1974. Studies of a surfactant and cholesteremia in the Maasi. *Am. J. Clin. Nutr*. 27: 464 - 466.

Mark, W. 2002. Food and Nutrition.[on line]. Healthy eating club, Australlia. Available:http://healthyeatingclub.com/info/articles/func foods/probiotics.htm. [20 June 2009].

Marshall, V. M. 1991. Gut-derived organisms for milk fermentations : Is Probiotics fact or fiction*? J. Chem. Technol. Biotechnol*. 51: 548–553.

Marteau, P., Flourie, B., Pochart, F., Chastang, C., Desjeux, J.F. and Rambaud, J.C. 1990. Effect of the microbial lactase (EC 3.2.1.23) activity in yoghurt on the intestinal absorption of lactose :An in vivo study in lactose deficient human. *Br. J. Nutr*. 64: 71-79.

Marteau, P., Pochart, P., Flourie, B., Pellier, P., Santos, L., Desjeux, J.F. and Rambaud, J.C. 1993. Effect of chronic ingestion of a fermented dairy product containing

Lactobacillus acidophilus and *Bifidobacterium bifidum* on metabolic activities of the colonic flora in humans. *Am. J. Clin. Nutr.* 52**:** 685–688.

Matsuzaki, T. and Chin, J. 2000. Modulating immune responses with probiotic bacteria. *Immunol. Cell Biol*. 78: 67-73.

Mayra-Makien, A. and Bigret, M. 1993. Industrial use and production of lactic acid bacteria. In: Salminen, S. and Von –Wright, A. (eds.). *Lactic Acid Bacteria*. Marcel Dekker, New York, pp 65–95.

McDonough, F.E., Hitchins, A.D., Wong, N.P., Wells, P. and Bodwell, C.E. 1987. Modification of sweet acidophilus milk to improve utilization by lactose-intolerant persons. *Am. J. Clin. Nutr.* 45: 570–574.

Mclntosh, G.H. 1996. Probiotics and colon cancer prevention. *Asia pacific J. Clin*. *Nutr.* 5: 48-52.

Medici, M., Vinderola C.G. and Perdigon G. 2004. Gut mucosal immunomodulation by probiotic fresh cheese. *Int. Dairy J.* 14: 611-618.

Metchinkoff, E. 1908. *The prolongation of life*. Putmans and sons, New York, 183p.

Michetti, P., Dorta, P.H., Wiesel, D., Brassart, E., Verda, M., Hereanz, C., Felley, N. and Porta, M. 1999. Effect of whey-based culture supernatant of *Lactobacillus acidophilus* (johnsonii) on Helicobacter pylori infection in humans. *Digestion*. 60: 203-209.

Midolo, P.D., Lambert, J.R., Hull, R., Luo, F. and Grayson, M.L. 1995. *In vitro* inhibition of *Helicobactor pylori* NCTC 11637 by organic acids and lactic acid bacteria. *J. Appl. Bact*. 79: 475-479.

Miettinen, M., Lehtonen, A., Julkunen, I. and Matikainen, S. 2000. *Lactobacilli* and *Streptococci* activate NF-Kappa B and STAT signaling path ways in human macrophages. *J. Iummunol*. 164: 3733-3740.

Miller, C.W., Nguyen, M.H., Rooney , M. and Kailasapathy, K. 2002. The influence of packaging materials on the dissolved oxygen content of probiotic yoghurt. *Packag. Techno*l. 15:133-138.

Mishra, C. and Lambert, J. 1996. Production of anti-microbial substances by probiotics. *.Asia Pacific J. Clin. Nutr*. 5: 20–4.

Morotomi, M. 1996. Properties of *Lactobacillus casei* shirota strain as probiotics. *Asia Pacific J. Clin. Nutr*. 5: 29-30.

Morteau, P., Vaerman, J.P., Bord, J.P., Brassart, D., Pochart, P., Desjeux, J.F. and Ramabaud, J.C. 1997. Effects of intrajejunal perfusion and chronic ingestion of *lactobacillus Johnsonii strain LAl* on serum concentrations and jejunal secretions of immunoglobulins and serum proteins in healthy humans. *Gasteroenterol. Clin. Biol*. 21: 293-298.

Mugula, J. K., Nnko, S. A. M., Narvhus, J. A., and Sørhaug, T. 2003 Microbiological and fermentation characteristics of togwa, a Tanzanian fermented food. International *J. Fd Microbiol*. 80: 187-199.

Munoz, P., Bouza, E., Cuenca-Estrella, M., Eiros, J.M., Perez, M.J., Sanchez-Somolinos, M., Rincon, C., Hortal, J. and Pelaez.T. 2005. *Saccharomyces cerevisiae fungemia*: An emerging infectious disease. *Clin. Infect. Dis.* 40(11): 1625–1634.

Nakamura, Y., Yamamoto, N., Sakal, K. and Takona, T. 1995. Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotenisin –i-converting enzyme. *J. Dairy Sci*. 78: 1253-1257.

NLAB. 2002. *The production of mixed starters containing Lactococci, Lactobacilli and Leuconostoc in commercial media*.[on line]. Research network on LAB Dairy research centre. Available:http://www.fsaa.ulaval.ca/stelawww /en/pdf/technofiche3a.pdf [22 March 2009].

Naidu, A.S., Bablack, W.K., and Clemens, R.A. 1999. Probiotic spectra of lactic acid bacteria(LAB). *Crit. Rev. Fd. Sci. Nutr*. 39: 13-126.

Namugumya, B.S. and Muyanja, C.M.B.K. 2009. Traditional processing, microbiological, physiochemical and sensory characteristics of *Kwete*, a Ugandan fermented maize based beverage. *Afr. J. Fd Agric. Nutr. Dev.* 9(4): 1046-1059.

Nanji, A.A., Khettry U. and Sadrzadeti, S.M.H. 1994. *Lactobacillus* feeding reduce endotoxemia and severity of experimental alcoholic liver disease. *Proc. Soc. Exp. Biol. Med*. 205: 243-247.

Nebesny, E., Zyzelewicz, D., Motyl, I. and Libudzisz, Z. 2007. Dark chocolates supplemented with *Lactobacillus* strains. *Eur. Fd Res. Technol*. 225: 33-42.

Niedzielin, K., Hubert, K. and Birkenfeld, B. 2001. A controlled, *double-blind, randomized study on the efficacy of Lactobacillus* plantarum 299V in patients with irritable bowel syndrome. *Eur. J. Gastroenterol*. 13(10): 1143-1147.

Nout, M.J.R. and Kiers, J.L. 2004. *Tempeh as a functional food*. Blackwell publishing, Netherlands, 233p.

Nout, M. J.R. and Ngoddy, P. O. 1997.Technological aspects of preparing affordable fermented complementary foods. *Fd Control.* 8: 279-287.

O'Sullivan, M. G. 1996. Metabolism of bifidogenic factors by gut flora – an overview. *Bulletin Int. Dairy Fed*. 313: 23–25.

Oatley J. T., Rarick M. D., Ji G. E. and Linz, J. E. 2000. Binding of aflatoxin B1 to *Bifidobacteria in vitro*. J. Fd Prot. 63: 1133-1136.

Oboh, G. 2006. Nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisae* and *Lactobacillus spp* solid media fermentation techniques. *J. Biotechnol*. 8(4): 58- 62.

Oboh, G. and Akindahunsi, A.A. 2003. Biochemical changes in Cassava products (flour & gari) subjected to *Saccharomyces cerevisae* solid media fermentation. *Fd Chem*. 82(4): 599-602.

Odunfa, S.A. 1985. *African Fermented foods.* Elsevier Applied Science Publishers, USA, 289p.

Okawa, T., Niibe, H. and Arai, T. 1993. Effect of LC9018 combined with radiation therapy on carcinoma of the uterine cervix. *Cancer*. 72: 1949-1954.

Oksanen, P.J., Salminen, S. and Saxelin, M. 1990. Prevention of traveler diarrhea by *Lactobacillus GG*. *Ann. Med*. 22: 53-56.

Olivares.M., Diaz-Ropero, M.B.S., Sierra, S., Lara-Villoslada, F., Fonolla , J., Navas, M..M., Rodríguez , M.J. and Xaus, J. 2007. Oral intake of *Lactobacillus fermentum* CECT5716 enhances the effects of influenza vaccination. *Nutrition*. 23: 254-260.

Onimawo, I.A., Nmerole, P.I., Idoko, R. and Akubor, P.I. 2003. Effects of fermentation on nutrient content and some functional properties of pumpkin seed (*Telfaria occidentalis*). *Pl. Fd. Hum. Nutr*. 59: 1-9.

Onwulata, C.I., Rao, R.D. and Vankineni, P. 1989. Relative efficiency of yoghurt, sweet acidophilus milk, hydrolysed lactose milk and a commercial lactase tablet in alleviating lactose maldigestion. *Am. J. Clin. Nutr*. 49: 1233-1237.

Osawa, T. 1998. Functional foods for disease prevention. In: Osawa, T., Shibamoto, T. and Terao, J.(eds.), *Recent progress on functional foods research in Japan*. American chemical society, Washington, pp. 2-9.

O'Toole, J. A . 2008. Cultures encapsulated with chocolate food products coated with chocolate and method of preparation[on line]. FoodInfo Online Innovations. Available: http://www.foodsciencecentral.com [13 April 2009].

Ouwehand, A. and Salminen, S. 1998. The health effects of cultured milk products with viable and non-viable bacteria. *Int. Dairy J.* 8: 749–758.

Ouwehand, A.C., Teija, K. and Paivi, R. 2004. Use of a probiotic *Bifidobacterium* in a dry food matrix, an *in vivo* study. *Int. J. Fd Microbiol.* 95(1): 103-106.

Oxman, T., Shapiea, M., Klein, R., Avazov, N. and Robinowitz, B. 2001. Oral administration of *Lactobacillus* induces cardioprotection. *J. Altern Complement Med*. 7(4): 345-354.

Oyetago, V.O. and Oyetago, F.L. 2005. Potential of probiotics as biotherapeutic agents targetins the innate immune system. *Afr. J. Biotech*. 4(2): 123-127.

Oyewole, O.B. 1991. Fermentation of cassava for *'lafun'* and *'fufu'* production in Nigeria. *Fd Lab. News*. 7(2): 29-31.

Page, A.L. 1982. *Methods of Soil Analysis*. Part II. American Society of Agronomists, Washington, D.C., 334p.

Parker, R.B. 1974. Probiotics, the other half of the antibiotic story. *Anim. Nutr. Hlth*. 29: 4-5.

Parvez, S., Malik, K.A., Ah Kang, S. and Kim, H.Y. 2006. Probiotics and their fermented food products are beneficial for health. *J. Appl. Microbiol*. 100: 1171-1185.

Pascual, M., Hugas, M., Jose, I.B., Josep, M.M. and Margarita, G. 1999. Lactobacillus salivarius CTC2197 *Prevents* Salmonella enteritidis *Colonization in Chickens.* Appl. Environ. Microbiol. *65(11): 4981-4986.*

Passeron, T., Lacour, J. P., Fontas, E. and Ortonne, J. P. 2006. Prebiotics and synbiotics : two promising approaches for the treatment of atopic dermatitis in children above 2 years. *Allergy*. 7: 431-437.

Patel, P., Parekh, T. and Subhash, R. 2008. Develpment of probiotic and symbiotic Chocolate mousse: A functional food. *Biotechnology*. 7(4): 769-774.

Payne, D. L., Welsh, J.D., Manion, C.V., Tsegaye, A. and Herd. L.D.1981. Effectiveness of milk products in dietary management of lactose malabsorbiton. *Am. J. Clin. Nutr*. 34: 2711- 2714.

Pearce, J. 1996. Effect of milk and fermented dairy products on the blood cholesterol content and profile of mammals in relation to coronary heart disease. *Int. Dairy J*. 6: 661.

Pelletier, X., Laure, S.B. and Donazzolo, Y. 2001. Hydrogen excretion upon ingestion of dairy products in lactose intolerant male subjects- importance of live flora. *Eur. J. Clin. Nutr*. 55: 509-512.

Perdigon , G., Alvarez, S., Rachid, M., Aguero, G. and Gobbato, N. 1995. Immune system stimulation by probiotics. *J. Dairy Sci* .78(7): 1597-1606.

Perdigon, G., Vintini, E., Alvarez, S., Medina, M. and Medici, M. 1999. Study of the possible mechanisms involved in the mucosal immune system activation by lactic acid bacteria. *J. Dairy Sci*. 82(6): 1108-1114.

Perkin-Elmer. 1982. *Analytical Methods for Atomic Absorption Spectrophotometry*. Perkin-Elmer Corporation, USA, 114p.

Peter H. G., Hennekens, H.C., Ma, J., Longcope, C. and Stampfer, J.M. 1996. Prospective Study of Sex Hormone Levels and Risk of Prostate Cancer. *J. Natl Cancer Inst.* 88(16): 1118-1126.

Peterson, E.S. 1971. *Microbiology of Food Fermentation.* AVI Publishers, Connecticut, 283p.

Picard, C., Fioramonti, J., Francois, A., Robinson, T., Neant, F. and Matuchansky, C. 2005. Review article: *Bifidobacteria* as probiotic agents – Physiological effects and clinical benefits. *Aliment. Pharmacol. Ther.* 22: 495–512.

Pillai, K.C. 2001. Shelf life of breadfruit (*Artocarpus altilis* (Park) Fosberg.). M.Sc. (Hort.) thesis, Kerala Agricultural University, Trichur, 85p.

Pisulewski, P. and Kostogrys, R.B. 2003. Functional properties of foods of animal origin and the methods of their assessment. *J. Fd. Nutr. Sci*. 1: 65-73.

Playne, M. 1994. Probiotic foods. *Fd Aust.* 46: 362-364.

Plessas, S., Pherson, L., Bekatorou, A., Nigam, P. and Koutinas, A.A. 2005. Bread making using kefir grains as baker's yeast. *Fd Chem.* 93: 585-589.

Pochart, P., Dewit, O., Desjeux, J. F. and Bourlioux, P. 1989. Viable starter culture, beta-galactosidase activity, and lactose in duodenum after yogurt ingestion in lactase-deficient humans. *Am. J. Clin. Nutr*. 49: 828–831.

Post , R.C.1996.Regulatory perspective of the USDA on the use of antimicrobials and inhibition in foods. *J. Fd Prot. Supp.*78-81.

Prajapathi, J.B., Shah, R.K., and Dave, J.M. 1987. Survival of *Lactobacillus acidophilus* in blended – spray dried preparations. *Aust. J Dairy Technol*. 42: 17-21.

Prasad, J., Paul, M., and Pramod, G. 2002. Heat and osmotic stress responses of probiotic *lactobacillus rhamnosus* HN001 (dr20) in relation to viability after drying. *Appl. Environ. Microbiol.* 69(2): 917–925.

Rafter, J., Bennett, M., Caderni, G., Clune Y., Hughes, R., Karlsson, C. P., Klinder, A.,O'Riordan, M ., O'Sullivan, C.G., Pool-Zobel, B., Rechkemmer, G., Roller, M., Rowland, I., Salvadori, M., Thijs, H., Loo,V. J., Watzl, B. and Collins, K. J. 2007. Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients. *Am. J. Clin. Nutr.* 85 : 488-496

Raimbault, O.A. and Tewe, O.O. 2001. Protein enrichment of sweet potato by solid substrate fermentation using four monoculture fungi. *Niger. J. Biotechnol*. 9(1): 1– 4.

Ramaswamy H,S. and Basak, S. 1992. Pectin and raspberry concentrate effects on the rheology of stirred commercial yoghurt. *J. Fd. Sci.* 57: 357-60.

Ranganna, S. 1986. *Handbook of Analysis and Quality Control of Fruit Vegetable Products*. Second edition, Tata McGraw Hill Pubishing Company Ltd., New Delhi, 594p.

Rangaswami, G. and Bagyaraj, D.J. 2000. *Agricultural Microbiology*. Second edition. Prentice Hall of India, Pvt. Ltd., New Delhi, 422p.

Rani, B., Khetarpaul, N. and Kumar, R. 1996. Development , acceptability and nutritional composition of food blends fermented with probiotic organisms. *Ann. Biosci.* 12 (1): 127-133.

Rani, B. and Khetarpaul, N. 1999. Probiotic fermentation of indigenously developed RSMT mixture: effect on antinutrients and digestibility of starch and proteins. *J. Fd Sci. Technol*. 36: 71-73.

Rani, B. and Khetarpaul, N. 1998. Probiotic fermentated food mixture: possible application in clinical and antidiarrhoea usage. *Nutr. Hlth*. 12(2): 97-105.

Rao, D.R. Chawan, C.B. and Pulsani, S.R. 1981. Influence of milk and thermophilus milk on plasma cholesterol levels and hepatic cholesterogenesis in rats. *J. Fd Sci*. 46: 1339-1341.

Rashid, U, H., Togo,K., Ueda, M. and Miyamoto, T .2007. Probiotic Characteristics of Lactic Acid Bacteria Isolated from Traditional Fermented Milk *'Dahi'* in Bangladesh. *Pak. J. Nutr*. 6 (6): 647-652.

Rastall, R.A. 2005. Modulation of the microbial ecology of the human colon by probiotics, prebiotics and synbiotics to enhance human health: An overview of enabling science and potential applications. *Microbiol. Ecol.* 52: 145–152.

Rautava, S., Kalliomaki, M. and Isolauri, E. 2002. Probiotics during pregnancy and breast feeding might confer immuno modulatory protection against atopic disease in the infant. *J. Allergy Clin. Immunol*. 109: 119-121.

Ravula, R.R. and Shah, N.P. 1998. Effect of acid casein hydrolyzates and cysteine on the viability of yogurt and probiotic bacteria in fermented frozen dairy desserts. *Aust. J. Dairy Technol.* 53: 174-179.

Reddy, B.S. and Riverson, A. 1993. Inhibitory effect of Bacillus longum on colon, mammary and liver carcinogenesis induced by 2 amino-3-methylimidazo(4,5..) quinoline, a food mutagen. *Cancer Res*. 53: 3914-3918.

Reid, G., Bruce, A.W. and Fraser, N. 2001. Oral probiotics can resolve urogenital infections. *FEMS Immunol. Med. Microbiol*. 30: 49-52.

Reid, G., Charbonnaeau, D. and Erb, J. 2003. Oral use of *Lactobacillus rhamnosus* GR-1 and Lactobacillus fermentum RC-14 signficantly alters vaginal flora: randomized placebo controlled in 64 healthy women. *FEMS Immunol. Med. Microbiol*. 35: 131-134.

Reuter, G. 1990. Bifidobacteria as components of yogurt-like products. *Bifidobacteria Microflora.* 9:107-118.

Rizkalla, S.W., Luo, J., Kabir, M., Chevalier, A., Pacher, N. and Slama, G. 2000. Chronic consumption of fresh but not heated yogurt improves breath hydrogen status and short-chain fatty acid profiles: A controlled study in healthy men with or without lactose maldigestion. *Am. J. Clin. Nutr.* 72: 1474–1479.

Roberfroid, M.B. 1998. Probiotic and symbiotic concepts and nutritional properties. *Br. J. Nutr*. 80: 197-202.

Roberfroid, M.B., VanLoo, J.A.E. and Gibson, G.R.1998. The bifidogenic nature of chicory inulin and its hydrolysis products. *J. Nutr. Sci*. 128: 11-13.

Rodrigues, K.L., Caputo, L.R.G., Carvalho, J.C.T., Evangelista, J. and Schneedorf. J.M. 2005. Antimicrobial and healing activity of kefir and kefir extract. *Int. J. Antimicrobial Agents.* 25: 404- 408.

Ross, R., Fitzgerald, G., Collins, J. K., Sullivan, G.C. and Stanton, C. 1999. Process for the manufacture of probiotic cheese. PCT International Patent Application, UK, Patent No. WO99/62348 AI.

Rowland, I. R. and Tanaka, R. 1993. The effects of transgalactosylated oligosaccharides on gut flora metabolism in rats associated with human faecal microflora. *J. Appl. Bact*. 74: 667–674.

Saarela, M., Lahteenma, L., Crittenden, R., Salminen, S. and Mattila-Sandholm, T. 2002. Gut bacteria and health foods-The European perspective. *Int. J. Fd Microbiol.* 78: 99–117.

Saavedra, J. M., Abi-Hanna, A., Moore, N. and Yolken, R. H. 2004. Long-term consumption of infant formulas containing live probiotic bacteria: Tolerance and safety. *Am. J. Clin. Nutr.* 79: 261–267.

Saavedra, J.M., Bauman, N.A. and Onug, I. 1994. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospitals for prevention of diarrhea and shedding of rotavirus. *Lancet*. 344:1046-1049.

Sadasivam, S. and Manikam, A. 1992. *Biochemical Methods for Agriculture Sciences*. Eighth edition. Wiley Eastern Ltd., New Delhi and Tamil Nadu Agricultural University, Coimbatore, 422p.

Sakamoto, I., Igarashi, M., Kimura, K., Takagi, A., Mixa, T. and Koya, T. 2001. Suppressive effect of *Lactobacillus gasseri OLL* 2716(LG21) on *Helicobacter pylori* infection in humans. *J. Antimicrob. Chemother*. 47: 709-710.

Sako, T., Matsumoto, K. and Tanaka, R. 1999. Recent progress on research and applications of non-digestible galactooligosaccharides. *Int. Dairy J*. 9: 69-80.

Salem, A. S., Gafour, W. A. and Eassawy, E. A. 2006. Probiotic milk beverage fortified with antioxidants as functional ingredients. *Egyptian J. Dairy Sci.* 34 (1): 23–32.

Salminen, M.R., Jarvinen, A., Saxelin, M., Tynkkgnen, S., Rautelin, H. and Valtonen, V. 2001. Increasing consumption of *Lactobacillus* GG as a probiotic and the incidence of lactobacilli bacteraemia in Finland. *Clin. Microbiol. Infection*. 7: 80-82.

Salminen, S. and Deighton, M. 1992. Lactic acid bacteria in the gut in normal and disordered states. *Dig. Dis. Sci*. 10: 227-238.

Salminen, S. and Marteau, P. 1997. Safety of probiotic lactic acid bacteria and other. *Adria Normandie Caen*. 12: 71–72.

Salminen, S., Bouley, M.C. and Boutron-Rualt, M.C. 1998 a. Functional food science and gastro-intestinal physiology and function. *Br. J. Nutr*. 80: 147-171.

Salminen. S., Von–Wright, A., Morelli, L., Marteau, P., Brassart, D., de Vos , W.M., Fonden, R., Saxelin, M., Collins, K., Mogensen, G., Birkeland, S.E. and Mattila-Sandholm, T. 1998 b. Demonstration of safety of probiotics: A review. *Int. J. Fd Microbiol.* 44(2): 93-106.

Samona, A. and Robinson, R.K. 1994. Effect of yogurt cultures on the survival of bifidobacteria in fermented milks. *J. Soc. Dairy Technol*. 47: 58–60.

Sanders, M. E., Walker, D. C., Walker, K. M., Aoyama, K. and Klaenhammer, T. R. 1996. Performance of commercial cultures in fluid milk applications. *J. Dairy. Sci.* 79: 943-955.

Sanders, M.E. 1998. Overview of functional foods: emphasis on probiotic bacteria. *Int. Dairy J*. 8: 341-347.

Sanders, M.E and Klaenhammer, T.R. 2001.The scientific basis of *Lactobacillus acidophilus* NCFM functionally as a probiotic. *J. Dairy Sci*. 84: 319-331.

Santos, M.C.R. and Soccol, C. R. 2003. Development of a new beverage with probiotic activity. In: Roussoi, S (ed.), *New horizons in biotechnology*. Kluwer Academic publishers, Netherlands , 364p.

Satterlee, L. D., Marshall, H. F. and Tennyson, J. M. 1979. *J. A. O. C. S.* 56:103-104.

Savaiano, D.A., ElAnouar, A. A., Smith, D.E. and Levitt, M. D. 1984. Lactose malabsorption from yogurt, pasteurized yogurt, sweet acidophilus milk, and cultured milk in lactase-deficient individuals. *Am. J. Clin. Nutr*. 40: 1219–1223.

Saviano, D.A., Ahou, E.I., Anouae, A., Smitu, D.E. and Levitt, M.D. 1984. Lactose malabsorption from yoghurt, pasteurized yoghurt, sweet acidophilus milk and cultured milk in lactase-deficient individuals. *Am. J. Clin. Nutr*. 40: 1219-1223.

Saxelin, M., Rautelin, H., Salminen, S. and Makela, P.H. 1996. The safety of commercial products with viable *Lactobacillus* strains. *Infect. Dis. Clin. Pract.* 5(5): 331-335.

Schaafsma, G., Meulig, W.J.A., Van- Dokkum, W. and Bouley, C. 1998. Effects of a milk product, fermented by *Lactobacillus acidophilus* and with fructo-

oligosaccarides added, on blood lipids in male volunteers. *Eur. J. Clin. Nutr.* 52: 436-440.

Schaeffer, A.J. and Stamey, T.A. 1977. Studies of introital colonization in women with recurrent urinary infections. IX. The role of antimicrobial therapy. *J. Urol*. 118: 221–224.

Schiffrin, E., Rochat, F., Lint-Amster, H., Aescllimann, J. and Donnet Hughes, A. 1995. Immunomodulation of blood cells following the ingestion of lactic acid bacteria. *J. Dairy Sci*. 78: 491-497.

Schiffrin, E.J., Brassart, D. and Serving, A.L. 1997. Immune modulation of blood leukocytes in human by lactic acid bacteria- Criteria for strain selection. *Am. J. Nutr*. 15: 205-207.

Schillinger, U. 1999. Isolation and identification of lactobacilli from novel-type probiotic and mild yoghurts and their stability during refrigerated storage. *Int. J. Fd Microbiol*. 47: 79-87.

Schrezenmeir, J. and De Vrese, M. 2001. Probiotics, probiosis and synbiosis: approaching a definition. *Am. J. Clin. Nutr*. 73(2): 361-364.

Schultz, Z. 2000. Probiotics and inflammatory bowel disease. *Am. J. Gastroenterol*. 1: 19-21.

Sekine, K., Watanbe-Sekine, E., Ohta, J., Toida, T., Tatsuki, T., Kawashima, T. and Hashimoto, Y. 1994. Induction and activation of tumoricidal cells *in vivo* by the bacterial cell wall of *Bifidobacterium infantis*. *Bifidobacteria Microflora*. 13: 65–77.

Semjonovs, P., Jasko, J., Auzina, L. and Zikmanis, P. 2008. The use of exopolysaccharide-producing cultures of lactic acid bacteria to improve the functional value of fermented foods. *J. Fd Technol.* 6(2): 101– 109.

Sendra, E., Fayos, P., Lario, Y., Fernández-Lopez, J., Sayas-Barbera , E. and Jose Perez-Alvarez, A.J. 2008. Incorporation of citrus fibers in fermented milk containing probiotic bacteria. *Fd Microbiol*. 25: 13-21.

Senok, A. C. 2009. Probiotics in the Arabian Gulf Region. *Fd Nutr. Res*. 53: 10-13.

Senok, A. C., Ismaeel, A. Y. and Botta1, G. A. 2005. Probiotics: facts and myths. *Clin. Microbiol. Infection.* 11: 958-966.

*Seo, G., Shimizu, K. and Kono, M. 1989. Inhibition of growth of some enteropathogenic strains in mixed cultures of *Streptococcus faecalis* and *Clostridium butyrium*. *Microbios.* 40: 151-160.

Shah , N.P. and Jelen.N. 1990. Survival of lactic acid bacteria and their lactases under acidic conditions. *J. Fd Sci*. 55: 506-509.

*Shah, N. P. 1997. Bifidobacteria: characteristics and potential for application in fermented milk products. *Milchwissenschaft.* 52:16-20.

Shah, N. P., Lankaputhra, W.E.V., Britz, M. and Kyle, W.S.A. 1995. Survival of L.acidophilus and Bifidobactrium bifidum in commercial yoghurt during refrigerated storage. *Int. Dairy J.* 5: 515-521.

Shah, N. P. 2001. Functional foods from probiotics and prebiotics. *Fd Technol*. 55(11): 46-53.

Shah, N.P. and Ravula, R. R. 2000. Influence of water activity of fermentation , organic acids production and viability of yoghurt and probiotic bacteria. *Aust. J. Dairy Technol.* 55: 127-131.

Shah, N.P., Ali, J.P. and Ravula, R.K. 2000. Populations of *Lactobacillus acidophilus*, *Bifidobacterium spp* and *Lactobacillus casei* in commercial fermented milk products. *Biosci. Microflora*. 19(1): 35-39.

Shah, N.P., Fedorak, R.N. and Jelen, D. 1992. Food consistency effects of Quarg in lactose absorption by lactose intolerant individuals. *Int. Dairy J*. 2: 257-269.

Shah, N.P., Lankaputhra, W.E.S., Britz, M. and Kyle, W.S.A. 1995. Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in commercial yoghurt during refrigerated storage. *Int. Dairy J*. 5: 515-521.

Shahani, K.M. and Chandran, R.C. 1979. Nutritional and healthful aspects of cultured and culture containing dairy food. *J. Dairy Sci*. 62: 1685-1694.

Sharma, A. and Khetarpaul, N. 1997. Effect of fermentation on phytic acid content and *invitro* digestibility of starch and protein of rice –black gram dhal-whey blends. *J. Fd Sci. Technol.* 34: 20-23.

Sharon, C.L. 2003. Nutritional and organoleptic qualities of value added products from breadfruit (*Artocarpus altilis* (Park) Fosberg.). M.Sc. (Home Science) thesis, Kerala Agricultural University, Trichur, 55p.

Shermak, M.A., Saavedra, J.M., Jackson, T.L., Huang, S.S., Baylers, T.M. and Perman, J.A. 1995. Effect of yoghurt on symptoms and kinetic of hydrogen production in lactose malabsorbing children. *Am. J. Clin. Nutr*. 62: 1003-1006.

Sheth, A.A. and Garcia-Tsao, G. 2008. Probiotics and liver disease. *J. Clin. Gastroenterol.* 42: 80-84.

Shu, Q., Zhou, J.S., Rutherfued, K.J., Birtler, M.J., Prasad, J. and Gopal, P.K. 1999. Probiotic lactic acid bacteria (*Lactobacillus acidophilus HN017, Lactobacillus rhamnosus HN001 and Bifidobacterium lactic HN019*) have no adverse effects on the health of mice. *Int. Dairy J*. 9: 831-836.

Sidhu, H., Milton, J., Allison, J.O., Chow ,M., Clark ,A. and Peck. B.A. 2001. Rapid reversal of hyperoxaluria in a rat model after probiotic administration of oxalobacter Formigenes. *J. Urol*. 166 (4): 1487-1491.

Siitonen, S., Vapaatola, H. and Salminen, S. 1990. Effect of *Lactobacillus GG* yoghurt in prevention of antibiotic associated diarrhea. *Ann. Med*. 22: 57-59.

Simon, G. L. & Gorbach, S. L. 1987. Intestinal flora and gastrointestinal function. In: Johnson, L. R. (ed.), *Physiology of the Gastrointestinal Tract*, Raven Press, New York, 1747p.

Sindhu, C.S. and Khetarpaul, N. 2002 .Effect of probiotic fermentation on antinutrients and in vitro protein and starch digestibilities of indigenously developed RWGT food mixture. *Nutr. Hlth*. 16:173-181.

Sindhu, C.S. and Khetarpaul, N. 2004. Development, acceptability and nutritional evaluation of an indigenous food blend fermented with probiotic organisms. *Nutr. Fd Sci*.35: 20-27.

Sindhu, C.S. and Khetarpaul, N. 2001. Probiotic fermentation of indigenous food mixture: effect on antinutrients and digestibility of starch and protein *J. Fd Composition Analysis*. 14: 601-609.

*Sindhu, C.S, Khetarpaul, N., and Sindhu, A. 2005. Effect of probiotic fermentation on carbohydrate and mineral profile of an indigenously developed food blend. *Acta Alimentaria*. 34: 41-47.

Singh, B. R. and Sharma, V.D. 1999. Characterization of broad-spectrum klebocin produced by *Klebsiella pneumoniae* subspecies *aerogenes. Indian J. Comp. Microbial. Immunol. Infect. Dis*. 20: 116-120.

Singh, B. R., Verma, J. C., Sharma, V. D. and Singh, V. P. 2004. Inhibitory effect of *Lactobacillus acidophilus* and *Klebsiella pneumoniae* on common enteropathogens isolated from food and clinical materials. *J. Fd Sci. Technol*. 5: 566-570.

Smith, H. W. 1965. Observations on the flora of the alimentary tract of animals and factors affecting its composition. *J. Path. Bact.* 89: 95-122.

Sobowale, A. O., Olurin, T. O., and Oyewole, O. B. 2007. Effect of lactic acid bacteria starter culture fermentation of cassava on chemical and sensory characteristics of fufu flour. Afr. J. Biotech. 6: 1954-1958.

Solga, S.F. 2003. Probiotics can treat hepatic encephalopathy. *Med Hypotheses*. 61(2): 307-313.

Solis, P. and Lemonnier, D. 1996. Induction of human cytokines by bacteria used in dairy foods. *Nutr. Res*. 13: 1127-1140.

Somoygi, N. 1952. Notes on sugar determination. *J. Biol. Chem*. 195: 19-23.

Song, Y.S., Frias, J., Martinez-Villaluenga, C., Vidal-Valdeverde, C., and de Mejia, G.E. 2008. Immunoreactivity reduction of soyabean meal by fermentaion, effect

on amino acid composition and antigenicity of commercial soy products. *Fd. Chem*. 108: 571-581.

Songisepp. E., Kullisaar, T., Hutt, P., Elias, P., Brilene, T., Zilmer, M. and Mikelsaar, M. 2004. A new probiotic cheese with antioxidative and antimicrobial activity. *J. Dairy Sci.* 87: 2017-2023.

Soni , S.K. and Sandhu, D.K.1990. Biochemical and nutritional changes associated with Indian Pubjabi Warri fermentation . *J. Fd Sci. Technol*. 27: 82-85.

Sotomayor , C., Frias , J., Vidal-Valverde , C., Fornal , J., Sadowska , J. and Urbano, G.1999. Lentil starch content and its microscopical structure as influenced by natural fermentation. *Starch - Starke.* 51(5): 152 – 156.

Sridar, R., Nguyen, M. and Kailasapathy, K. 2003. Studies on the effect of encapsulation on the survival of probiotic microorganisms unde high acid and bile conditions. *J. Fd Sci. Technol.* 40: 458-460.

Stadlbauer.V., Rajeshwar, P., Mookerjee, P., Hodges, S., Gavin, A. K., Davies, N. A. and Jalan, R. 2008. Effect of probiotic treatment on deranged neutrophil function and cytokine responses in patients with compensated alcoholic cirrhosis. *J. Hepatol.* 48(6): 945-951.

Stamey, T. A. 1973. The role of introital enterobacteria in recurrent urinary infections. *J. Urol*. 109: 467-472.

Stanton, C., Gardiner, G. and Meehan, H. 2001. Market potential for probiotics. *Am. J. Clin. Nutr*. 73: 476–483.

Stanton. C., Gardiner, G., Lynch, P. B., Collins, J.K., Fitzgerald, G. and Ross. R.P. 1998. Probiotic cheese. *Int. Dairy J.* 8: 491-496.

Steinkraus, H.K. 1995. *Handbook of indigenous fermented foods.* Marcel Dekker, Inc, New York, 776p.

Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P. and Kailasapathy, K. 2000. Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *Int. J. Fd. Microbiol.* 62: 47-55.

Sunny, R, E., Otunola, E., and Iwakun, B. 2004. An evaluation of some quality parameters of a laboratory-prepared fermented groundnut milk. *Eur. Fd Res. Techno*l. 218(4): 452-455.

Sutas, Y., Hurme, M. and Isolauri, E. 1996. Down regulation of anti-co3 antibody induced IL-4 production by bovine caseins hydrolysed with *Lactobacillus GG* derived enzymes. *Scand. J. Immunol*. 98: 216-224.

Suvarna, V.C. and Boby, V.U. 2005. Probiotics in human health: A current assessment. *Curr. Sci*. 88(11): 1744-1748.

Szajewska, H., Kotowska, M., Mrukowicz, J.Z., Armanska, M. and Mikolajczyk, W. 2001. Efficacy of *Lactobacillus GG* in prevention of nosocomial diarrhea in infants. *J. Pediatr.* 138: 361–365.

*Tabera, J., Frias, J., Estrella, I., Villa, R. and Vidal-Valverde, C. 1995. Natural fermentation of lentils: influence of time, concentration and temperature on protein content, trypsin inhibitor activity and phenolic compound content. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* . 201: 587-591.

Takahashi, C., Kikucbi, N. and Katou, N. 1995. Possible anti-tumor promoting activity of componenets in soybean fermented food, Natto: Effect on gap junctions, intercellular communication. *Carcinogenesis*, 16(3): 471-6.

Takano, T., Koichiro, A., Murota, I., Hayakawa, K., Mizutani, T. and Mitsuoka, T 1985. Effects of feeding sour milk on longevity sad tumorigenesis in mice and rats. *Bifidobacteria Microflora* 4: 31-33.

*Takiguchi, R., Ohe, M. and Miyamoto, M. 1985. Effect of fermented milk administration on faecal microflora and faecal excertion of demented senile patients who are habitually using purgative. *Bifidus-Flores Fructus Semina.* 8: 7-14.

Tamai, Y., Yoshimitsu, N., Watanabe, Y., Kuwabara, Y. and Nagai, S. 1996. Effects of milk fermented by culturing with various lactic acid bacteria and yeast on serum cholesterol level in rats. *J. Ferment. Bioengng*. 81: 181-182.

Tanaka, R. and Matsumoto, K. 1998. Recent progress on prebiotics in Japan, including galacto-oligosaccharides. *Bulletin Int. Dairy Fed*. 336: 21-27.

Teixeira, P., Castro, H. and Kirby**,** R. 1995. Spray drying as a method for preparing concentrated cultures of *Lactobacillus bulgaricus*. *J. Appl. Bacteriol*. 78: 456-462.

Tissier, H. 1906. Traitement des infections intestinales pae la mebode de la flore bacterienne de' lintestin. *CR. Soc. Biol*. 60: 359-361.

Topping, D.L. 1996. Short chain fatty acids produced by intestinal bacteria. *Asia Pacific J. Clin. Nutr*. 5: 15-19.

Tramer, J. 1973. Yogurt cultures. *J. Soc. Dairy Technol*. 26: 16-18.

Trapp, C.L., Chang, C.C., Halpern, G.M., Keen, C.L., and Gershwin, M.E. 1993. The influence of chronic yogurt consumption on populations of young and elderly adults. *Int. J. Immunol. Ther*. 9: 53–64.

Trepanier, G., Simmard, R. E. and Lee, B. E. 1991. Lactic acid bacteria in relation to accelerated maturation of Cheddar cheese. *J. Fd Sci*. 56:1238-1240.

Trinchieri, G. 1994. Interleukin-12: A cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. *Blood.* 84: 4008–4027.

Tsangalis, D., Ashton, J. F., McGill, A. E. J. and Shah, N. P. 2003. Biotransformation of isoflavones by bifidobacteria in fermented soymilk supplemented with D-glucose and L-cysteine. *J. Fd Sci.* 68**:** 623–631.

Tsen , J ., Yeu-Pyng, L., Hui-Ying, H., King, V. and An-Erl. 2007. Accelerated storage testing of freeze-dried immobilized *Lactobacillus acidophilus* fermented banana media. *J. Fd Processing Preservation*. 31: 688-701.

Tuohy, K. M., Hollie, M., Smejkal, W. C. and Gibson, R. G. 2003. Using probiotics and prebiotics to improve gut health. *Drug Discovery Today.* 8: 692-700.

Tyopponen, S., Esko, P. and Mattila-Sandholm, T. 2003. Bioprotectives and probiotics for dry sausages. *Int. J. Fd Microbiol*. 83: 233-244.

UNFAO/WHO [United Nations. Food and Agriculture Organization of the United Nations/World Health Organization]. 2002. UNFAO/WHO home page [on line]. Available: http://ftp.fao.org/es/esn/food/wgreport2.pdf [04 May. 2009].

Urga, K., Fite, A. and Birastu, E. 1997. Effect of natural fermentation on natural and antinutritional factors of *tef(Ergrastis tef). Ethiopian J. Hlth. Devlpt*. 2: 61-66.

Usman, P. and Hosono, A. 1999. Bile tolerance, taurocholate deconjugation and binding of cholesterol by *Lactobacillus gasseri* strains. *J. Dairy Sci*. 82: 956-961.

Varela-Moreiras, G., Antoine, J. M., Ruiz-Roso, B. and Varela, G. 1992. Effects of yogurt and fermented-then-pasteurized milk on lactose absorption in an institutionalized elderly group. *J. Am. Nutr*. 11: 168–171.

Varshey, S.C. 2002. Role of functional foods in diets. *Indian Fd. Ind*. 21: 41-43.

Vaughan, E.E., Mollet, B. and De vos, V.M. 1999. Functionality of probiotics and intestinal *lactobacilli:* Light in the intestinal tract tunnel. *Curr. Opin Biotechnol*. 10: 505-510.

Vedamuthu, E. R. 1974. Cultures for buttermilk, sour cream and yogurt with special comments on acidophilus yogurt. *Cult. Dairy Prod. J*. 9:16-17.

Vega, E.Z., Glatz, B.A., and Hamond, E.G. 1988. Optimisation of banana juice fermentation for the production of microbial oil. *Appl. Environ. Microbiol*. 54: 748-752.

Vergin, F. 1954. Anti- und Probiotika. *Hippokrate.* 25: 116–119.

Wang, H.L. and Hesseltine, C.W. 1981. Use of Microbial Cultures: Legume and Cereal Products. *Fd Technol*. 35 (1): 79 -81.

Wang, N. F. 2007. Evaluation of peanut flour fermented with lactic acid bacteria as a probiotic food**.** *Fd Sci. Technol. Int.* 13: 469-475.

Whorwell, P.J., Altringer, L., Morel, J., Bond, Y., Charbonneau, D., O'Mahony, L., Kiely, B., Shanahan, F. and Quigley, E.M. 2006. Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel syndrome. *Am. J. Gastroenterol.* 101: 1581–1590.

Williams, M., Pehu, E. and Ragasu, C. 2006. Functional foods: Opportunities and challenges for developing countries [on-line]. Agricultural and rural development. Available: http://www.worldbank.org/rural.

Wisselink H.W., Weusthuis, R.A., Eggink, G., Hugenholtz ,J. and Grobben, G. J. 2002. Mannitol production by lactic acid bacteria: a review. *Int. Dairy J.* 12: 151-161.

Witthuhn, R.C., Schoeman, T. and Britz, T. J. 2005. Characterisation of the microbial population at different stages of Kefir production and Kefir grain mass cultivation. *Int. Dairy J.* 15: 383-389.

Wojtas, P. A., Hansen, L.T. and Paulson, A.T. 2007. Microstructural studies of probiotic bacteria-loaded alginate microcapsules using standard electron microscopy techniques and anhydrous fixation. *Fd Sci. Technol*. 41: 101-108.

Wolf, B.W., Wheeler, K.B., Ataya, D.G. and Garleb, K.A. 1998. Safety and tolerance of *Lactobacillus reuteri* supplementation to a population infected with human immunodeficiency virus. *Fd. Chem. Toxicol*. 36: 1085-1094.

Yadav, H., Jain, S. and Sinha, P. R. 2008. The effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* on gastropathic consequences in diabetic rats. *J. Med. Fd*. 11(1): 62-68.

Yasui, H., Nagoaka, N. and Mike, A. 1992. Detection of bifidobacterium strains that induce large quantities of IgA. *Microbiol. Ecol. Hlth Dis*. 5: 155-162.

Yoo B. and Lee C.M. 1993. Thermoprotective effect of sorbitol on proteins during dehydration. *J. Agric. Fd Chem*. 41: 190-192.

Yoon, H.J., Kang,S., Mheen, T.I., Ahn, S.J., Lee, H.J., Kim, T.K., Park, S.C., Kho, H.Y., Kang, H.K. and Park, Y.H. 2000. *Lactobacillus kimchii sp*. a new species from kimchi. *Int. J. Systematic Evolutionary Microbiol*. 50: 1789–1795

Yoon, K.Y, Edward , E., Woodams, E. and Yong, D. 2006. Hang Production of probiotic cabbage juice by lactic acid bacteria. *Bioresource Technol*. 97: 1427-1430.

Yoon, K.Y., Edward, E.W. and Hang, D.Y. 2004. Probiotication of tomato juice by lactic acid bacteria. *J. Microbiol*. 42(4): 315-318.

Young, G.P. 1996. Prevention of colon cancer: Role of short chain fatty acids produced by intestinal flora. *Asia Pacific J. Clin. Nutr*. 5: 44-47.

Zamora, A. F. and Fields, M. L. 1979. Nutritive quality of fermented cow peas and chick pea. *J. Fd Sci*. 44: 234-236.

Zhao, H.Y., Wang, H.J., Lu, Z. and Xu, S. Z. 2004. Intestinal microflora in patients with liver cirrhosis. *Chinese J. Digestive Dis*. 5: 64-67.

Zhou, J.S., Shu, Q., Rutherfued, K.J, Prasad, J., Birtler, M.J. and Gopal, P.K. 2000. Safety assessment of potential probiotic lactic acid bacterial strains(*Lactobacillus rhamnosus HN001, Lactobacillus acidophilus HN017 and Bifidobacterium lactic* HN019)in BALB/C mice. *Int. J. Fd. Microbiol*. 56: 87-96.

Ziemer, C.J. and Gibson, G.R. 1998. An overview of probiotics, prebiotics and synbiotics in the functional foods concept: perspectives and future strategies. *Int. Dairy J*. 8: 473-479.

Zubillaga, M., Weil, R., Postaire, E., Goldman, C., Caro, R. and Boccio, J. 2001. Effect of probiotics and functional foods and their use in different diseases. *Nutr. Res*. 21: 569-579.

* Originals not seen

Appendices

APPENDIX- I

Score card for organoleptic evaluation of banana based food mixtures

Sl.No	Quality parameters	Description	Score	1	2	3	4	5
1	Appearance	Excellent Good Fair Poor Very poor	5 4 3 2 1					
2	Colour	Excellent Good Fair Poor Very poor	5 4 3 2 1					
3	Flavour	Excellent Good Fair Poor Very poor	5 4 3 2 1					
4	Texture	Excellent Good Fair Poor Very poor	5 4 3 2 1					
5	Taste	Excellent Good Fair Poor Very poor	5 4 3 2 1					
6	Overall acceptability	Excellent Good Fair Poor Very poor	5 4 3 2 1					

Date: Name:

Signature:

APPENDIX- II

Score card for organoleptic qualities of banana based food mixtures

Characteristics	**Score**		
	1	2	3
Appearance			
Taste			
Flavour			
Colour			
Texture			
Overall acceptability			

Name of the judge:
Date:

9 point Hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Signature:

STANDARDISATION AND QUALITY EVALUATION OF BANANA BASED PROBIOTIC FERMENTED FOOD MIXTURES

By

Sharon.C.l.

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

DOCTOR OF PHILOSOPHY IN HOME SCIENCE

Faculty of Agriculture

Kerala Agricultural University

Department of Home Science

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2010

1ABSTRACT

The study entitled "Standardisation and quality evaluation of banana based probiotic fermented food mixtures" was undertaken with the objective to standardise indigenous food mixtures based on banana flour with probiotic fermentation with *Lactobacillus acidophilus* and to evaluate the nutritional factors, organoleptic qualities and storage stability of the food mixtures.

Probiotic characteristics like acid and bile tolerance and antimicrobial activity of *L.acidophilus* MTCC 447 showed an acid tolerance ranging from pH 2.0 - 9.0 , a bile tolerance of three per cent and antagonistic activity against enteropathogens viz *Salmonella enteritidis, E.coli, Bacillus cereus and Staphylococcus aureus.*

The foods selected for developing the probiotically fermented food mixtures were banana (Nendran), defatted soya flour, green gram flour, ripe mango, papaya and tomato. From the 56 combinations tried, 14 fermented food mixtures with *L. acidophilus* MTCC 447 were selected statistically by applying Kendall's coefficient of concordance.

All the 14 selected food mixtures contained 60-70 per cent banana flour, 20 per cent defatted soy flour / green gram flour and 10-20 per cent fruit pulps.

For all the treatments variables of fermentation were optimised as 25g of the food mixture (substrate), pH 4.5, inoculum 300µl (119 x 10^6 cfu /ml), temperature of incubation 37 ºC and time of incubation 24 hours.

All the fermented foods along with unfermented controls were freeze dried. Constituents like titrable acidity (2.59 g lactic acid / 100g), protein (7.82g/100g), iron (6.48mg/100g), thiamine (0.0726 mg/100g) and riboflavin (0.535 mg/100g) were significantly high in fermented food mixtures. *in vitro* digestibility of starch (82.109 per cent) and protein (85.85 per cent) were also significantly high in fermented food mixtures. Total viable count of *L. acidophilus* ranged from 9.13 to 9.46 log cfu/g. Mean

score of overall acceptability of fermented products were between 7.9-8.0 in a 9 point hedonic scale.

From 14 fermented food mixtures, six fermented food mixtures were statistically selected considering all the quality aspects by geometric mean score. The selected food mixtures T_1, T_2, T_3, T_7, T_8 and T_9 along with their respective controls were packed in metallised poly ester / poly ethylene laminate pouches and kept for storage studies under ambient conditions for a period of six months.

From the six fermented food mixtures with maximum shelf life qualities, three fermented food mixtures were statistically selected by applying geometric mean score. The treatments with maximum geometric mean score were T_1 (70 per cent banana flour, 20 per cent defatted soy flour, 10 per cent mango), T_3 (60 per cent banana flour, 20 per cent defatted soy flour, and 10 per cent tomato pulp) and T_8 (60 per cent banana flour, 20 per cent defatted soy flour,10 per cent mango and 10 per cent tomato pulp).In all the selected three treatments, viable count of *L.acidophilus* ranged from 8.84 to 9.12 log cfu/g after six months of storage. This viable count was within the desired level of probiotic organisms recommended

Substrate composition was modified by adding sucrose, sorbitol, wheat bran and skimmed milk powder to T_1, T_3 and T_8.The level of these four ingredients were standardised as five per cent in T_3, with maximum viable counts of *L.acidophilus* ranging from 9.45 to 9.54 log cfu/g. Thus five treatments (T_3 + sucrose 5 %, T3 + 5% sorbitol, T_3 + 5% wheat bran and T_3 + 5 % skimmed milk powder) was subjected to quality evaluation and shelf life studies.

After modifying the substrate, food mixture T_3S (with added sucrose at 5 per cent level) showed high acceptability and an increase in the viable count of *L.acidophilus* after storage, when compared to T_3 (control).T_3SB (with added sorbitol at 5 per cent level) was comparable to that of T_3 (control) in any aspect. T_3W (with added wheat bran at 5 per cent level) was also comparable to T_3 (control) but with an increase in the total

viable count .T_3SK (with added skimmed milk powder at 5 per cent level) showed an increase in all the nutrients, acceptability and viable counts. Hence, these four food mixtures (T_3S. T_3SB, T_3 W and T_3 SK) can also be recommended as good probiotic food mixtures.

The viable count of *L. acidophilus* in the developed probiotic food mixtures at the expiry period (after six months of storage) ranged between 95 to 210 x 10^7 cfu/g and in five grams the viability ranged between 475 to 1040 x 10^7 cfu. This was within the recommended level of the probiotic organism to assure health benefits. Since the fermented food mixtures were slightly acidic in taste, it can be used with acidic foods like buttermilk, fruit juices etc to enhance their acceptability. The cost of the developed food mixtures ranged between Rs 530 to Rs 550 for 400 grams.