

# **DEVELOPMENT OF A GERIATRIC FOOD AND EVALUATION OF ITS FUNCTIONAL POTENTIAL**

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**Dissertation**

**Submitted in the partial fulfillment of the requirement for**

**the award of the degree of**

**MASTER OF TECHNOLOGY**

**IN**

**BIOTECHNOLOGY**



By

**JHILMIL TANDON**

601204011

Under the guidance of

**Dr. ABHIJIT GANGULI**

Associate Professor

DEPARTMENT OF BIOTECHNOLOGY

THAPAR UNIVERSITY, PATIALA (PUNJAB)-147004

JULY 2014

## CANDIDATE'S DECLARATION

I, hereby declare that the work presented in the thesis entitled "Development of a Geriatric Food and evaluation of its Functional Potential" in the partial fulfillment of the requirement for the award of the degree of Master of Technology in Biotechnology, Department of Biotechnology, Thapar University, Patiala, is an authentic record of my work during the period of one year from July 2013 to June 2014, under the guidance of Dr. Abhijit Ganguli, Associate Professor, Thapar University, Patiala. I have not submitted the matter embodied in this thesis for the award of any other degree or diploma.

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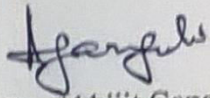
*Jhilmil.*

JHILMIL TANDON  
(601204011)

## CERTIFICATE

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This is to certify that the thesis entitled "**Development of a Geriatric Food and evaluation of its functional potential**" submitted by Jhilmil Tandon in partial fulfillment of the requirement for the award of Degree of Master of Technology in Biotechnology to Thapar University, Patiala, is a record of student's own work carried out by her. The report has not been submitted for the award of any other degree or certificate in this or any other University or Institute.

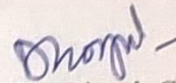


Dr. Abhijit Ganguli

Associate Professor, Supervisor

Department of Biotechnology, TU

Patiala

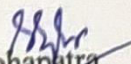


Dr. Dinesh Goyal

Professor & Head

Department of Biotechnology, TU

Patiala



Dr. S.K. Mohapatra

Dean

Academic Affairs,

Thapar University

Patiala

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**Jhilmil Tandon**  
**(601204011)**

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## **ABSTRACT**

The prevalence of degenerative or infectious diseases tends to increase with a growing ageing population. Malnutrition in the elderly is often underdiagnosed in persons over the age of 65. Supplementation of micro-nutrients, antioxidants and probiotics is widely advocated to improve the immune response and prevent chronic disease. The study aimed to develop a fermented food product 'Yoghurt' having bioactive substances along with functional properties. *Lactobacillus acidophilus* and *Streptococcus thermophilus* were used to produce the functional food product. Carrot yoghurt was prepared in laboratory with optimized conditions of inoculum (1%) and sugar (5 gm). Shelf life studies were also conducted upto 14 days at refrigerated storage. In comparison to control yoghurt, the titratable acidity, pH, syneresis and shelf life was found more significant. The pH values of the samples ranged between 4.31-4.33 which was reasonably suitable for yoghurt. Nutritional analysis was also conducted. Presence of Riboflavin and Vitamin C was studied which satisfied the Recommended Dietary Allowance of Geriatric population. Presence of  $\gamma$ -Amino butyric acid (GABA) at concentration of 6.04 mM/ gm was also confirmed by Thin Layer Chromatography. Due to the presence of Beta-carotene (5130.12  $\mu$ g/100 gm) in yoghurt, antioxidant properties of yoghurt increases significantly. Sensorial analysis based on 9 point hedonic scale scored adequately for general acceptability for elderly. Extraction and quantification of Exopolysaccharide was performed. Production of EPS was quite significant (4.40 mg/100gm of yoghurt). The results of this study suggest a potential of applicability of this functional food for adults as well as for other age groups in terms of addressing nutritional deficiencies. However, clinical trials are recommended prior to application of this functional food.

## **LIST OF ABBREVIATIONS**

Abs	Absorbance
ATP	Adenosine Triphosphate
cfu	Colony Forming Units
GABA	$\gamma$ -aminobutyric acid
LAB	Lactic Acid Bacteria
TLC	Thin Layer Chromatography
Sec	Seconds
Min	Minutes
NADP	Nicotinamide adenine dinucleotide phosphate
M	Molar
mg	Milligram
ml	Millilitre
mM	Millimolar
MRS	De Mamm, Rogosa and Sharpe
RDA	Recommended Dietary Allowance
R <sub>f</sub>	Retardation factor
rpm	Revolutions per minute
$\mu$ g	Microgram
$\mu$ l	Microlitre
EPS	Exopolysaccharide
°C	Degree Celsius
CY	Carrot Yoghurt

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# **INTRODUCTION**

## Chapter 1. INTRODUCTION

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“Geriatrics” is the branch of medicine dealing with the physiologic characteristics of aging and the diagnosis and treatment of diseases affecting the aged. Geriatric nutrition applies nutrition principles to delay effects of ageing and disease. Advances in science and medicine as well as improved living standards have led to a steady increase in life expectancy. Ageing is associated with increased susceptibility to degenerative or infectious diseases, which may be exacerbated by a poor nutritional status. Age-related factors, such as physiological changes of the gastrointestinal (GI) tract, changes in dietary habits and the reduction of the functionality of the immune system, force severe shifts in the intestinal microbiota composition (Rampelli *et al.*, 2013). Nutrition is an important determinant of health in persons over the age of 65. Malnutrition in the elderly is often under diagnosed. Ageing is associated with a variety of physiological, behavioral and socio-economic changes. All of these factors could negatively affect nutritional behavior and dietary intake. Thus, older people are at an increased risk of impaired nutrition and health status. Physical conditions common in older persons, such as, disability, medication-induced anorexia, restrictive diets, gastrointestinal diseases, and metabolic disorders (diabetes mellitus and renal failure), all affect nutritional intake and metabolic demand. As with the young, depression is common in the elderly, but may present a typically as multiple somatic complaints, cognitive impairment, or weight loss. It is perhaps the most common cause of reversible weight loss in older persons. In addition, depression often impairs the motivation to obtain, prepare, and consume nutritionally adequate food.

### 1.1 Geriatric Health in India

In India, the cutoff point for geriatric age is 60 years. Geriatrics grows increasingly important as modern medicine and rising standard of living have lengthened life expectancy, resulting in increased proportion of aged persons in society. Elderly persons show lot of variation in age-related physiological decline and medical disorders. India is in a phase of demographic transition and trends reveal that population of elderly is growing faster than general population. India has thus, acquired the label of “an ageing nation” with 8.3% of its population being more than 60 years old. In India, Malnutrition is a consequence of anorexia, since in elderly, the food intake

decreases to a level below the energy demand. Monotonous diet, lack of sufficient fresh food/fruits and vegetables, inadequate intake of important micronutrients, contribute to malnutrition. The adverse health consequences of malnutrition are impaired muscle function, decreased bone mass, immune dysfunction, anemia, reduced cognition, delayed wound healing, and delayed recovery from surgery, increased risk of falls, disability and mortality. Degenerative diseases such as cardiovascular and cerebrovascular disease, diabetes, osteoporosis and cancer, which are among the most common diseases affecting older persons, are all diet-affected. Increasingly in the diet/disease debate, the role that micronutrients play in promoting health and preventing non-communicable disease is receiving considerable attention. Nutrient deficiencies (Vitamin C, Vitamin B<sub>2</sub>, Calcium) are often common in elderly people due to a number of factors such as their reduced food intake and a lack of variety in the foods they eat (Gopal *et al.*, 2013). Gamma-aminobutyric acid (GABA), which is a ubiquitous non-protein amino acid, is a major inhibitory neurotransmitter (Krogsgaard-Larsen, 1989; Mody *et al.*, 1994). GABA is involved in the regulation of cardiovascular functions, such as blood pressure and heart rate, and plays a role in the sensations of pain and anxiety (Mody *et al.*, 1994). There must be the consumption of GABA-enriched foods in the diet of elderly. Another factor is the price of foods rich in micronutrients, which further discourages their consumption. Compounding this situation is the fact that the elderly often suffer from decreased immune function, which contributes to this group's increased morbidity and mortality. Other significant age-related changes include the loss of cognitive function and deteriorating vision, all of which hinder good health and dietary habits in old age.

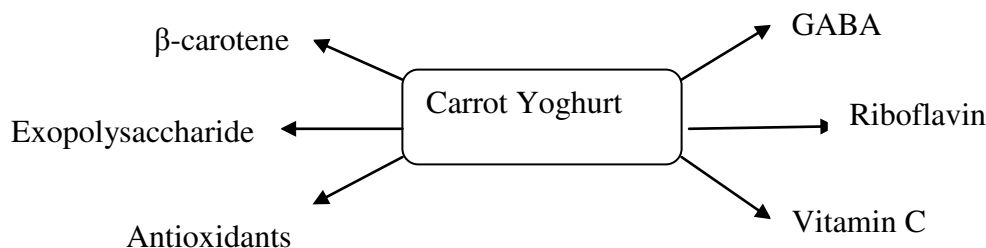
So far, no study has explored whether a functional yoghurt supplemented with *Streptococcus thermophilus* and *L. acidophilus* along with carrot juice could meet and address the nutritional (Vitamin C, Vitamin B<sub>2</sub>, Calcium) needs of the Geriatric people. Yoghurt was anticipated to be the food matrix on account of the simplicity, economics of preparation, general acceptability, consumer awareness and all the benefits of a fermented milk product. However, the consistency of yoghurt suits the denture problems frequented in the elderly.

## **1.2 Yoghurt**

Yoghurt is defined as a product resulting from milk by fermentation with a mixed starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*.

Probiotics, especially *Lactobacillus acidophilus*, strains have increasingly been incorporated into food products as dietary adjuncts due to their purported health benefits. In some countries less traditional microorganisms, such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii*, are sometimes mixed with the starter culture (McKinley, 2005). Although fermented milk products such as yoghurts were originally developed simply as a means of preserving the nutrients in milk, it was soon discovered that, by fermenting with different microorganisms, an opportunity existed to develop a wide range of products with different flavors, textures, consistencies and more recently, health attributes.

Yoghurt's nutritional profile has a similar composition to the milk from which it is made but will vary somewhat if fruit, cereal or other components are added. Fruit enhancement plays a considerable role in yoghurt consumption and also increases the consumer acceptability (Cakmakci *et al.*, 2012). In efforts to offer variety and competition in the market, new research is currently in progress on the use of carrot juice in yoghurt industry (Schieber *et al.*, 2002 and Simova *et al.*, 2004). Carrot (*Daucus carota*), which is one of the important root vegetables, has appreciable levels of carotenoids and dietary fibers. It is an important source of natural antioxidants having anticancer activity. Carrot juice is rich in vitamin A, beta-carotene, minerals and it is easier to digest than raw or cooked vegetables. Carotenoids are important nutrients in fruit and vegetable based products to human health and nutrition because of their provitamin A function and antioxidant properties (Knockaert *et al.*, 2012). In spite of their low percentage in milk, carotenoids are involved in the sensory properties of dairy products via their colorant and antioxidant properties (Agabriel *et al.*, 2007). Current developments in new ready-to-drink beverages are based on drinks containing combinations of dairy and fruit juices with added bioactive components (Zulueta *et al.*, 2010). The use of carrot with yoghurt is advantageous due to its antibacterial and antifungal properties. In addition, carrot is safe for public health and used as vitaminized food supplement.



**Figure 1: Pictorial representation of health benefits of Carrot Yoghurt**

### **1.3 OBJECTIVES**

Nutrient deficiencies are frequent amongst older people, the nutrients, which are of particular concern in older people, are Calcium, Vitamin A and Vitamin B12. There is evidence that the requirement for certain nutrients increase with ageing, for example Vitamins B6, B12, K and folate are some of the nutrients that have a protective role to play in the ageing process. Elderly are more vulnerable to infections because their immune system has been weakened by many age related factors.

As deliberated earlier, very few studies in India have emphasized foods especially for the elderly. Yoghurt is an ideal Geriatric food because it can be taken in both liquid as well as semi-solid form and is sensorially acceptable. Also, elderly have more sensitive colons or who no longer produce much lactase, yoghurt is also a valuable food. Moreover, there is considerable evidence regarding other potential benefits of probiotics (if administered through food) in reducing the risk of cancer, influencing immunomodulatory features, preventing food allergies, counteracting hypercholesterolemia, and alleviating the symptoms of lactose intolerance. The benefits derived from a regular intake of probiotics foods are also correlated to their ability to inhibit pathogens and protect humans from gastrointestinal diseases.

Accordingly, the following objectives were framed:

- 1) Development of food matrix for delivery of high value nutraceuticals suitable for the elderly.
- 2) Optimization of culture and raw materials like inoculums size, sugar, etc.
- 3) Viability, Stability and Shelf life studies of the food developed.
- 4) Analysis of Physiochemical and Functional Attributes.

# **REVIEW OF LITERATURE**

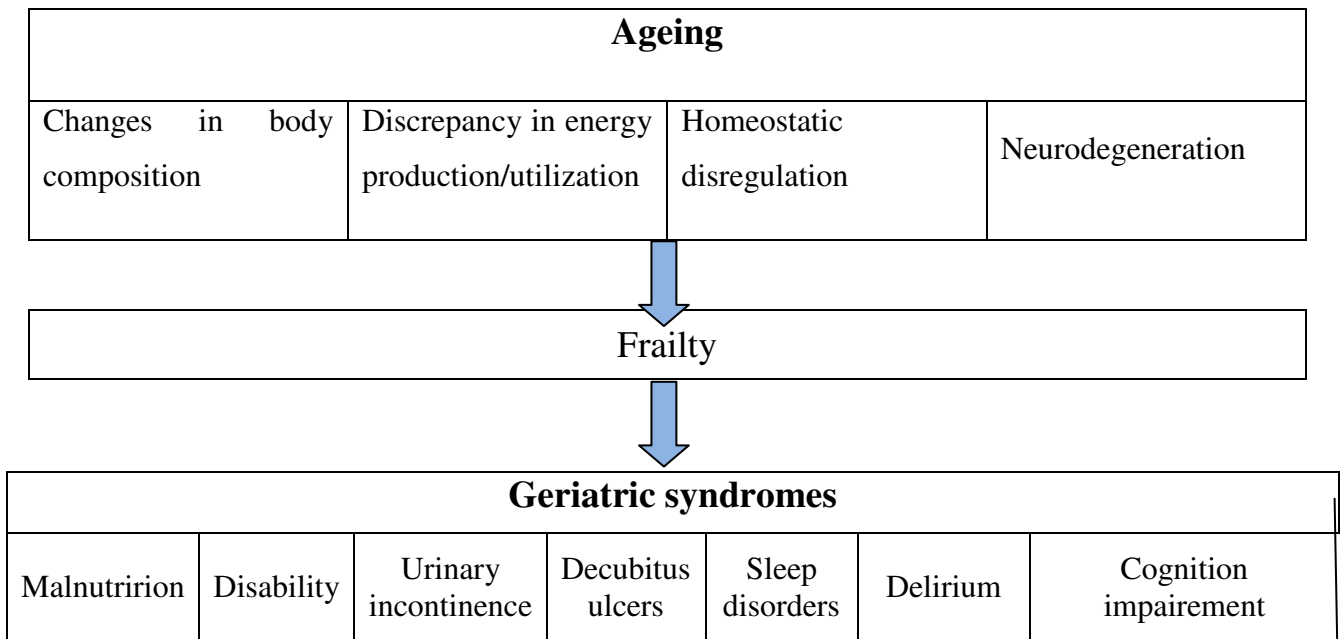
### 2.1 Health issues in Geriatrics

World Health Organization defines “health” as a state of complete physical, mental, social and spiritual well-being and not merely the absence of disease or infirmity. Ageing is related to the deterioration of some physiological and health functions, thus, elderly people are more susceptible to various diseases and illnesses. Nutrition plays an important role in preventing diseases and promoting recovery from illnesses (Morley *et al.*, 1992). Poor nutrition and malnutrition occur in 15 to 50 percent of the elderly population. But the symptoms of malnutrition (weight loss, disorientation, lightheadedness and loss of appetite) can easily be mistaken for illness or disease. Changes associated with normal aging increase nutritional risk for older adults. Ageing is characterized by diminished organ system reserves, weakened homeostatic controls, and increased heterogeneity among individuals, influenced by genetic and environmental factors. Nutritional needs of the older individual are determined by multiple factors, including specific health problems and related organ system compromise; an individual's level of activity, energy expenditure, and caloric requirements; the ability to access, prepare, ingest, and digest food; and personal food preferences.

In India, the elderly people suffer from dual medical problems, i.e., both communicable as well as non-communicable (Gopal *et al.*, 2013). The immune defense system in particular is known to be adversely affected by the aging process, and there is strong evidence that a poorly functioning immune system can contribute to decreased disease resistance and reduced life expectancy in the elderly (Wayne *et al.*, 1990). India is in a phase of demographic transition and trends reveal that population of elderly is growing faster than general population. There has been a sharp increase in the number of elderly persons between 2001 and 2011 and it has been projected that by the year 2050, the number of elderly people would rise to about 324 million (Gopal *et al.*, 2013).

The composition of the intestinal microbiota in older people (>65 years) is extremely variable between individuals, and differs from the core microbiota and diversity levels of younger adults. A feature of the ageing process is immunosenescence, evidenced by persistent NF-kB-mediated

inflammation and loss of naive CD4+ T cells (Franceschi *et al.*, 2000). The microbiota is pivotal for homeostasis in the intestine, and chronic activation of the innate and adaptive immune system is linked to immunosenescence (Guigoz *et al.*, 2008). Deterioration in dentition, salivary function, digestion and intestinal transit time may affect the intestinal microbiota upon ageing. Nutrient deficiencies (Vitamin C, Vitamin B2, Calcium) are often common in elderly people due to a number of factors such as their reduced food intake and a lack of variety in the foods they eat (Gopal *et al.*, 2013).



**Figure2: Pathophysiology of health issues in geriatrics**

## 2.2 Lactic acid bacteria

Lactic acid bacteria (LAB) constitute a diverse group of Gram-positive bacteria, characterized by some common morphological, metabolic and physiological traits. They are anaerobic bacteria, non-sporulating, acid tolerant and produce mainly lactic acid as an end product of carbohydrate fermentation. This bacterial group contains both rods (Lactobacilli and Carnobacteria) and cocci (Streptococci). Different species of Lactic acid bacteria (such as *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Aerococcus*, *Lactobacillus*) have adapted to grow under widely different environmental conditions. They are found in gastrointestinal tract of various animals, dairy products, seafood products, soil and on some plant surfaces (Ring, 1998). Although lactic acid

bacteria are not dominant in the normal intestinal microbiota, several trials have been undertaken to induce an artificial dominance of lactic acid bacteria (Verschuere, 2000).

Lactic acid produced by fermentation is a biological process which is known for centuries. Many different cultures in various parts of the world carry out fermentation to improve the storage qualities and nutritive value of perishable foods such as milk, vegetables, meat fish and cereals. LAB those produces such type of fermentation products plays an important role in preserving foods. In developed world, lactic acid bacteria are mainly associated with fermented dairy products such as cheese, buttermilk, and yoghurt. The uses of dairy starter cultures gave rise to an industry during this century. Today, an increasing number of health food and so- called functional foods as well as pharmaceutical preparation are promoted with health claims based on the characteristics of certain strains of lactic acid bacteria. The concept of the group name ‘lactic acid bacteria’ was created for bacteria causing fermentation and coagulation of milk, and defines as those which produce lactic acid from lactose. The family name Lactobacteriaceae was applied by Orla-Jensen (1919) to a physiological group of bacteria producing lactic acid alone or acetic and lactic acids, alcohol and carbon dioxide.

**Table 1: Few families of order Lactobacillales**

<b>Family:</b> <i>Lactobacillaceae</i>	
<b>Genus:</b> <i>Lactobacillus</i>	
Strictly fermentative with complex nutritional requirements; grow in and are associated with many different habitats; aciduric or acidophilic, produce pH 4.0 in foods containing a fermentable carbohydrate; often suppress growth or kill there bacteria; important in fermented food manufacture (dairy, meat, beer and wine) as well as spoilage.	
<i>L. plantarum</i>	Synonyms: <i>L. arizonensis</i> , <i>Sreptobacterium plantarum</i> , <i>L. arabinosus</i> , <i>L. plantari</i> . Often found in association with cabbage products and with Cheddar cheeses; can cause spoilage of pickles, acidity and off-flavour in wine. Ferments lactose, maltose and mannitol; grows predominantly at 15 °C.
<i>L. casei</i>	Synonyms: <i>L. casei casei</i> , <i>Streptobacterium casei</i> , <i>Bacillus casei a</i> , <i>Bacillus a</i> . Found in association with milk products. Ferments lactose, maltose and

	mannitol. Forms short to long rods with square ends. Probiotic, used in yoghurts.
<i>L. helveticus</i>	Synonyms: <i>L. suntoryeus</i> , <i>L. helveticum</i> , <i>Plocamobacterium helveticum</i> , <i>Bacillus casei e</i> , <i>Bacillus e</i> . Belong to Thermobacteria; important probiotic, used in production of fermented dairy products, can cause spoilage of beer.

<b>Family:</b> <i>Streptococcaceae</i>	
<b>Genus:</b> <i>Lactococcus</i>	
<i>Lactic streptococci</i> . The genus was proposed by Schleifer et al. in 1985 to re-classify some species of the genera <i>Streptococcus</i> and <i>Lactobacillus</i> . Lactococci are homofermentative and exclusively produce L-lactate; generally found on plants and the skins of animals (Casalta E, 2008). Lactococci are used widely in dairy industry. The principle concern is reliability and stability of their starter cultures because many of the desirable traits of the lactococci are plasmid dependent and can be unstable.	
<i>S.thermophilus</i>	Synonyms: <i>Streptococcus salivarius</i> subsp. <i>thermophilus</i> , is found in fermented milk products, and is generally used in the production of yoghurt, yoghurt and cheese that contain live cultures of <i>S. thermophilus</i> are thought to be beneficial to health.

### 2.3 Probiotic Lactic Acid Bacteria

The term ‘probiotics’ as described by Fuller (1989) as ‘a preparation or product containing viable, defined micro-organisms in sufficient numbers that alter the microflora in a compartment of the host and that exerts health effects in the host’. Lactic acid bacteria (LAB) including *Lactobacillus* spp. such as *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, and *Bifidobacterium* spp. have been recognized to be important in the maintenance of the human intestinal microbial ecosystem. Member of LAB are widely-used probiotics in fermented foods and beverage industry and also contributes to the sensory qualities of the food and also in the prevention of spoilage. Improvement in intestinal disorders and lactose intolerance, altered vitamin content of milk, antagonism against various pathogenic organisms and antimutagenic and anti-carcinogenic

activities are some of the health benefits of LAB. Probiotic organisms has been reported to overcome symptoms of lactose intolerance (DeVerse *et al.*, 1992), improved immune function, cholesterol lowering potential (Noh *et al.*, 1997), antimutagenic activity (Lankaputhra and Shah, 1998) and treatment of diarrhea (Guandilini *et al.*, 2000). LAB are capable of producing several different polysaccharides, which can be found in the cytoplasm as carbon and energy sources, as components of the bacterial cell wall, or as external appendages of the cell. Use of exopolysaccharide producing yoghurt starter cultures is gaining popularity among yoghurt manufacturers (Wacher-Rodarte *et al.*, 1993; Hess, Roberts, & Ziegler, 1997). Gassem, Schmidt, and Frank (1995) and Griffin, Morris, and Gasson (1996) reported that polysaccharide-producing yoghurt bacteria were important determinants of yoghurt viscosity and texture. These starter cultures improve the viscosity of yoghurt leading to resistance to mechanical damage (Tamime & Deeth, 1980).

## **2.4 Biochemistry of Lactic Acid Bacteria**

Lactic acid bacteria are chemotrophic, they find the energy required for their metabolism from the oxidation of chemical compounds. The oxidation of sugars constitutes the principle energy producing pathway. Lactic acid bacteria assimilate sugars by either a homofermentative or heterofermentative pathway.

### **2.4.1 Homofermentative Metabolism of Hexoses**

Homofermentative bacteria transform nearly all of the sugars they use, especially glucose into lactic acid. The homofermentative pathway includes a first phase of all the reactions of glycolysis that lead from hexose to pyruvate. The terminal electron acceptor in this pathway is pyruvate which is reduced to lactic acid. In fermentation, pyruvate is decarboxylated to ethanal, which is the terminal electron acceptor, being reduced to ethanol.

### **2.4.2 Heterofermentative Metabolism of Hexoses**

Bacteria using the heterofermentative pathway, which includes *Leuconostoc* (the most important bacterium in ecology), use the pentose phosphate pathway. In this pathway, NADPH is generated as glucose is oxidized to ribose 5-phosphate. This five-carbon sugar and its derivatives are

components of important biomolecules such as ATP, CoA, NAD<sup>+</sup>, FAD, RNA and DNA. NADPH is the currency of readily available reducing power in cells (NADH is used in the respiratory chain). This pathway occurs in the cytosol.

After being transported into the cell, a glucokinase phosphorylates the glucose into glucose 6-P (glucose 6-phosphate). Its destination is completely different from the glucose 6-P in the homofermentative pathway. Two oxidation reactions occur: the first leads to gluconate 6-P and the second, accompanied by a decarboxylation, forms ribulose 5-P. In each of these reactions a molecule of NADP<sup>+</sup> is reduced. Ribulose 5-P can then be epimerized either to ribose 5-P or to xylulose 5-P. Xylulose 5-P is then cleaved into acetyl-phosphate and glyceraldehydes 3-phosphate. The glyceraldehyde 3-phosphate is metabolized into lactic acid by following the same pathway as in the homofermentative pathway. The acetyl-phosphate has two possible destinations, depending on environmental conditions. This molecule can be successively reduced into ethanal and ethanol, in which case the molecules of the coenzyme NADPH formed during the two oxidation reactions of glucose at the beginning of the heterofermentative pathway, are reoxidized. This reoxidation is essential for regenerating the coenzymes necessary for this pathway. The final products are then lactate and ethanol.

The acetyl-phosphate can produce acetate (acetic acid) through the enzyme acetate kinase. This reaction also yields a molecule of ATP. The final products of this pathway are then lactate and acetate. Bacteria of the genus *Leuconostoc* preferentially produce lactate and ethanol in a slightly aerated environment and lactate and acetate in an aerated environment.

Figure 3: Homofermentative metabolism of LAB

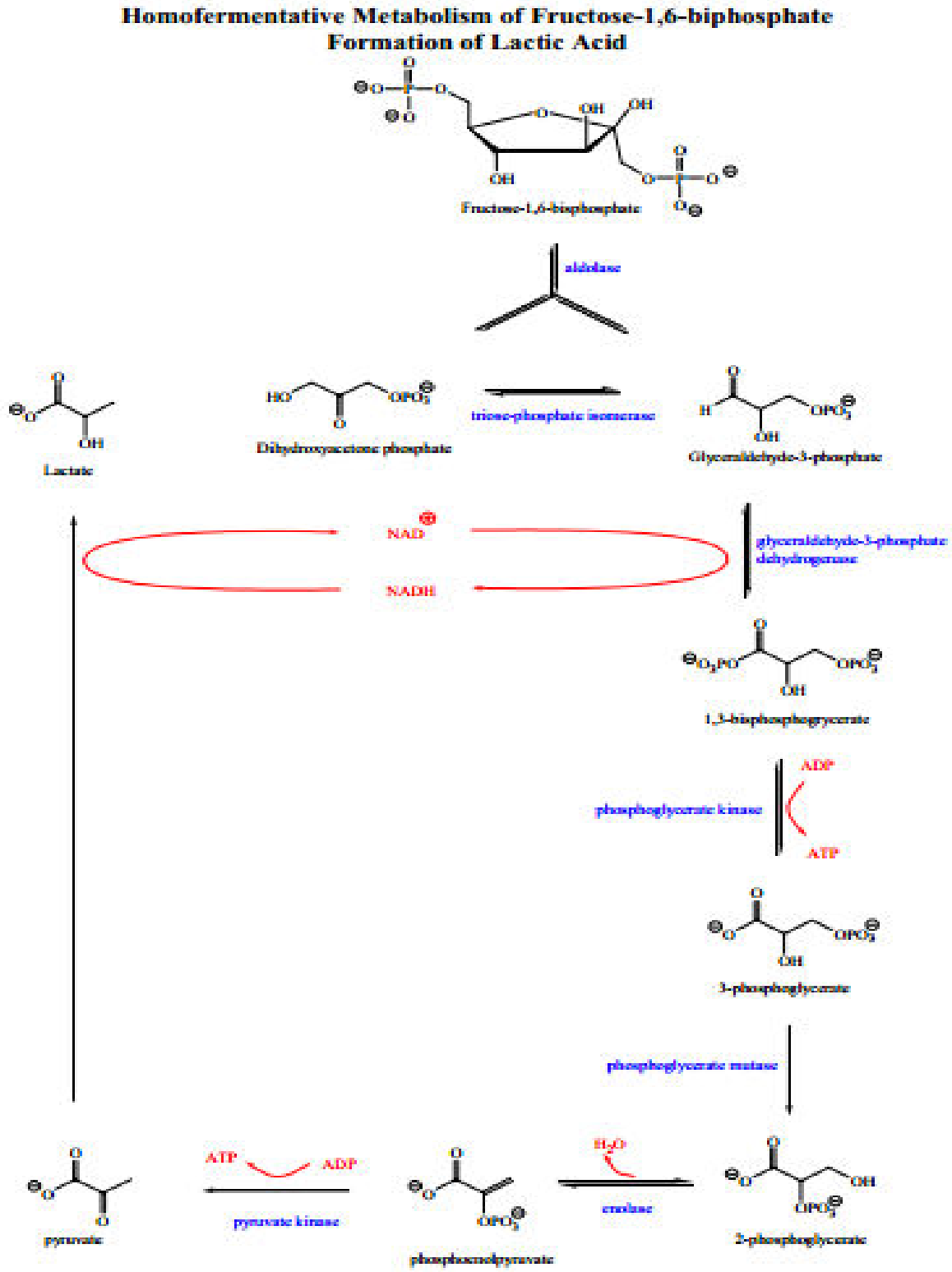
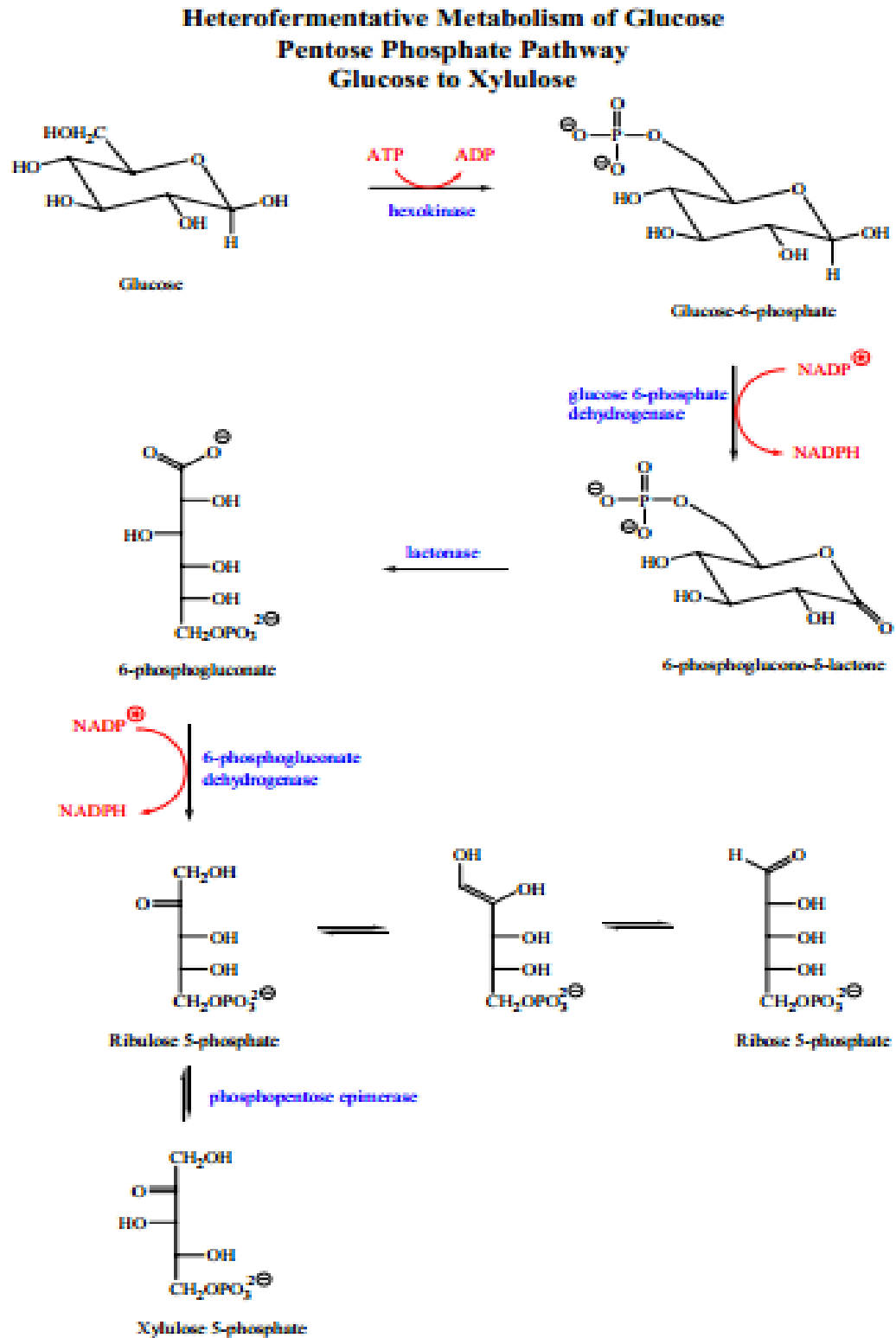
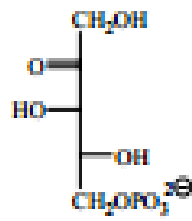
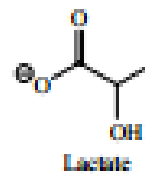
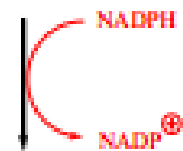
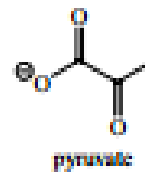
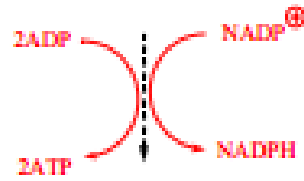
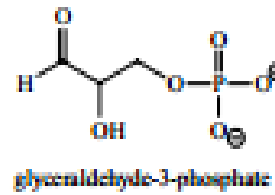
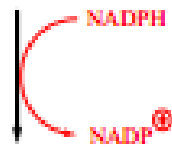
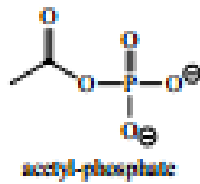


Figure 4: Heterofermentative metabolism of LAB





Xylulose 5-phosphate



same as homofermentative pathway

## 2.5 Probiotics

One manner in which modulation of the gut microbiota composition has been attempted is through the use of live microbial dietary additions, as probiotics. The word probiotic is translated from the Greek meaning ‘for life’. An early definition of probiotic was given by Parker (1974) as: ‘Organisms and substances which contribute to intestinal microbial balance.’ However, this was subsequently refined by Fuller (1989) as: ‘a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.’ A probiotic would therefore incorporate living microorganisms, seen as beneficial for gut health, into diet.

Probiotics has a long history. In fact, the first records of intake of bacterial drinks by humans are over 2000 years old. However, at the beginning of this century probiotics were first put onto a scientific basis by the work of Metchnikoff at the Pasteur Institute in Paris. Metchnikoff (1907) observed longevity in Bulgarian peasants and associated this with their elevated intake of soured milks. During these studies, he hypothesized that the normal gut microflora could exert adverse effects on the host and that consumption of certain bacteria could reverse this effect. Metchnikoff refined the treatment by using pure cultures of what is now called *Lactobacillus delbrueckii* subsp. *bulgaricus*, which, with *Streptococcus salivarius* subsp. *thermophilus*, is used to ferment milk in the production of traditional yoghurt.

Subsequent research has been directed towards the use of intestinal isolates of bacteria as probiotics (Fernandes *et al.*, 1987). Over the years many species of micro-organisms have been used. They mainly consist of lactic acid producing bacteria but also *Bacillus spp.* and fungi such as *Saccharomyces spp.* and *Aspergillus spp.*

Despite the very widespread use of probiotics, the approach may have some difficulties. The bacteria used are usually anaerobic and do not relish extremes of temperature. To be effective, probiotic must be amenable to preparation in a viable form at a large scale. During use and under storage the probiotic should remain viable and stable, and be able to survive in the intestinal ecosystem, and the host animal should gain beneficially from harbouring the probiotic. It is therefore proposed that the exogenous bacteria reach the intestine in an intact and viable form, and establish there and exert their advantageous properties. In order to do so, microbes must overcome a number of physical and chemical barriers in the gastrointestinal tract. These include

gastric acidity and bile acid secretion. Moreover, on reaching the colon the probiotics may be in some sort of stressed state that would probably compromise chances of survival.

## **2.6 Health Benefits of *Lactobacillus acidophilus***

*Lactobacillus acidophilus* offers a range of health benefits which include: providing immune support for infections and cancer are a healthy replacement of good bacteria in the intestinal tract following antibiotic therapy, reducing occurrence of diarrhea in humans (children and adults), aiding in lowering cholesterol, improving the symptoms of lactose intolerance. Anti-tumor effect of *L.acidophilus* was reported by Goldin and Gorbach (1984). Anticarcinogenic effect of *L.Acidophilus* may be due to direct removal of procarcinogens and activation of body's immune system. Animal studies have shown that dietary supplementation with *L.acidophilus* decrease the number of colon cancer cells in a dose dependant manner (Rao *et al.*, 1999).

## **2.7 Milk based fermented foods**

Milk and milk based products are consumed most popularly due to their nutritive value. For the same reason, milk is easily spoiled by pathogenic microorganisms; hence fermentation of milk using lactic acid bacteria is preferred for prevention. Lactic acid bacteria convert milk sugar lactose into lactic acid and produces antibacterial substance bacteriocin to suppress spoilage bacteria. *Dahi or curd* is most popular traditional Indian fermented product prepared by fermentation of milk by lactic acid bacteria. *Dahi* differs from yoghurt in its use of mixed starters of mesophilic lactococci. A principle flavor inducing metabolite is diacetyl, which is appreciated more by people of South Asian origin compared to the acetaldehyde flavour in yoghurt. LAB species isolated from fermented milk products include *Streptococcus cremoris*, *S. lactis*, *S. thermophilus*, *L. acidophilus*, *L. helveticus*, *L. plantarum*, *L. farciminis*, *L. brevis*, *Lactococcus lactis* subsp. *cremoris*, *L. casei* subsp. *casei* and *L. bifementans*. There are reports that LAB isolated from dahi can be used to cure intestinal disease such as diarrhea (Agarwal, 2001), intake of dahi has anti-cholesteremic (Singh *et al.*, 2012), anti-diabetic and angiotensin-converting enzyme inhibition effect.

## 2.8 Traditional fermented food: *Yoghurt*

Yoghurt is defined as a product resulting from milk by fermentation with a mixed starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*. However, in some countries, including Australia, other suitable lactic acid bacteria are permitted for use as starter cultures. As a result, some yoghurt manufacturers use *Lactobacillus helveticus* and *Lactobacillus jugurti* for yoghurt manufacture

### Nutritional Consideration

**Table 2: Some typical water-soluble vitamin contents of skim milk and low-fat yoghurt**

Vitamin ( $\mu\text{g}$ )	Milk (100 gm)	Yoghurt (100 gm)
Thiamin, B1	42	40
Riboflavin, B2	180	200
Pyridoxin, B6	42	46
Cobalamin, B12	0.4	0.2
Folic Acid	0.3	4.1
Nicotinic Acid	180	125
Pantothenic Acid	370	380
Biotin	1.6	2.6

Homogenization of milk is used to effect stabilization of the lipid phase against separation by gravity. Upon homogenization, the fat-globule membrane is destroyed, and the fat globule is vulnerable to attack by lipase, which is naturally present in milk. Because of this, the milk is pasteurized to inactivate lipase before homogenization. Heat Treatment is given typically at 85 °C for 30 min to destroy all pathogenic bacteria, to inactivate all the enzymes that may be present in milk, including lipase, to destroy most of the spoilage-causing bacteria, including thermodurics. The mixture is cooled to 45 °C and inoculated at a level that varies from 0.5 to 5%. The maximum amount recommended is 5%. This level will cause very rapid acid production, but leads to defects in aroma, and a large amount of culture must be prepared. There has been a phenomenal increase in the production of fermented milks in developed countries. Yoghurt is a very popular flavorful and healthful dairy product in India. Its production and

consumption is growing continuously due to its therapeutic properties beside its high nutritive value (Karagul *et al.*, 2004). The health promoting properties of live lactic acid bacteria in yoghurt include protection against gastrointestinal upsets, enhanced digestion of lactose by maldigesters, decreased risk of cancer, lower blood cholesterol, improved immune response and help the body assimilate protein, calcium and iron (Perdigeon *et al.*, 1998; Marona and Pedrigo, 2004). In efforts to offer variety and competition in the market, new research is currently in progress on the use of carrot juice in yoghurt industry (Schieber *et al.*, 2002 and Simova *et al.*, 2004).

## **2.9 Functional properties of Carrot**

Carrot has beta-carotene which is a powerful antioxidant and protects us against the destructive action of the free radicals. Carrot act as potent antioxidants, not only preventing vitamin A deficiency but also cancer and other diet related human diseases. It reduces the enzymes that convert precarcinogens to carcinogens. It may also enhance the immune system, protect against stroke, high blood pressure, Osteoporosis, cataracts, arthritis, heart disease, bronchial asthma and urinary tract infections (Beom *et al.*, 1998; Sun *et al.*, 2001; Seo and Yu, 2003). Some health promoting functions of carotenoids include Pro-vitamin A activity, inhibition of cancer, prevention of cardiovascular diseases, prevention of muscular degeneration and immune-enhancement. Carrots have potassium in it which helps to balance the high levels of sodium associated with hypertension and keeps blood pressure under control.

## **2.10 Application of functional food to improve conditions of Vitamin deficiency**

Vitamin C (ascorbic acid) plays a major role in human metabolism ranging from the synthesis of collagen, carnitine and norepinephrine to a large number of antioxidant activities (Padayatty, 2003). Humans are dependent on dietary sources, mainly fruits and vegetables as they are unable to synthesize Vitamin C. Even in high income countries, elderly population-based studies have reported blood levels of Vitamin C in the range indicating deficiency in around 1 in 5 men and 1 in 9 women in low income groups (Cahill, 2009). There are scarce data on Vitamin C deficiency in resource-poor countries where Vitamin C deficiency might be expected to be more prevalent. A systematic review found that a third of reproductive stage, women in resource-poor settings

had dietary Vitamin C intakes below the Estimated Average Requirements (EAR) rising to nearly 50% in Africa and South East Asia (Torheim, 2010).

Riboflavin is continuously excreted from the urinary of healthy individuals. A deficiency of riboflavin can be primary-poor vitamin sources in one's daily diet-or secondary, which may be a result of conditions that affect absorption in the intestine, the body not being able to use the vitamin, or an increase in the excretion of the vitamin from the body (Brody, 1999).

### **2.11 Influence of functional food on Exopolysaccharide and $\gamma$ -Aminobutyric acid (GABA) production**

Many functional characteristics of lactic acid bacteria (LAB) are responsible for their historical and modern use in food production. One such characteristic that has recently generated much interest is polysaccharide production. LAB are capable of producing several different polysaccharides, which can be found in the cytoplasm as carbon and energy sources, as components of the bacterial cell wall, or as external appendages of the cell. Exopolysaccharides (EPS) are polysaccharides external to the cell, either attached to cells or excreted as free polysaccharides (Cerning, 1990; Cerning *et al.*, 1992; Nakajima *et al.*, 1990).

$\gamma$ -Aminobutyric acid (GABA) is a non-protein amino acid that is widely distributed in nature and produced from lactic acid bacteria through fermentation (Manyam *et al.*, 1981; Lu *et al.*, 2008). GABA is produced primarily by the decarboxylation of L-glutamic acid and catalyzed by the enzyme, glutamate decarboxylase (Mayer *et al.*, 1990). GABA has several well-known physiological functions involving neurotransmission and induction of antihypertensive effects, diuretic effects, and tranquilizer effects (Jakobs *et al.*, 1993). In several studies, the screening of GABA-producing lactic acid bacteria and the production of food enriched with GABA by lactic acid bacteria has been reported (Nomura *et al.*, 1998; Lu *et al.*, 2008; Sun *et al.*, 2009; Lee *et al.*, 2010). *L. lactis* is a lactic acid bacterium some strains of which have been reported to produce GABA from glutamate in fermented milk (Nomura *et al.*, 1998; Inoue *et al.*, 2003; Hayakawa *et al.*, 2004).

# **MATERIALS AND METHODS**

### 3.1 Chemicals and Media

All the chemicals and reagents used were of the highest analytical grade and purchased from Sigma Aldrich (St. Louis, MO, USA) unless otherwise specified. Standard media components were purchased from Fisher Scientific (USA) or Sigma Aldrich (St. Louis, MO, USA) and Himedia (Mumbai, India).

### 3.2 Microorganisms

*Streptococcus thermophilus* (NDRI, Karnal) was used as a starter culture for yoghurt production. *Lactobacillus acidophilus* ATCC43121 was also used. Working cultures of the strains were maintained on De Man Rogosa and Sharpe (MRS) Agar (Himedia, Mumbai) at 4-6 °C and *Streptococcus thermophilus* isolation Agar. Routine cultures were done by incubation of cultures in media at 37 °C under shaking conditions.

### 3.3 Gram Staining

Bacterial smear from actively growing cells were spread on a glass slide and heat fixed. Smear was flooded with filtered crystal violet for 10 sec and then washed briefly in water to remove excess crystal violet. Later, it was flooded with Gram's iodine for 10 sec and washed briefly with water. Smear was decolorized with acetone until the moving dye front had passed the lower edge of the section and washed immediately in tap water. Counterstaining was carried out with safranin for 15 sec and washed with water to remove the excessive stain. Finally, samples were visualized under microscope at different magnification. The morphology of strains was studied up to 100 x magnifications microscopically (Nikon, 200 N, Japan).

### 3.4 Enumeration of culture purity

Tenfold serial dilutions of 1 gm yoghurt sample were prepared with 9 ml of 0.85% saline solution and 1 ml of the three highest dilutions was pour-plated in duplicate. Colonies counts of strains were enumerated on ST agar and MRS agar under anaerobic conditions at 30 °C for 72 hours. Colony Forming Units (CFU) containing 30-350 colonies were calculated per gram of sample.

### **3.5 Preparation of Carrot Juice**

Two hundred and fifty grams of good quality carrots (purchased from local market, Patiala) were washed under cold water and were scrubbed with a vegetable brush. Carrots were placed in a blender or food processor and were processed until finely chopped or mashed. Small amount of water was added for proper mashing. The puree was poured into the large measuring cup. The juice was strained using a strainer, stored under refrigerator and used within 2 hrs.

### **3.6 Optimization of Carrot juice, Inoculum size and Sugar**

Carrot juice, sugar and inoculums size was optimized as described by Salwa *et al.* (2004) and Park *et al.* (2005). 10%, 15% and 20% Carrot Juice was selected for further studies and inoculums size of 1% was optimized for yoghurt preparation, 5 gm of sugar was optimized for yoghurt preparation.  $\beta$ -carotene levels were measured as described in British Pharmacopeia 2003.

### **3.7 Preparation of Carrot Yoghurt**

Plain and sugary yoghurt samples were produced both with and without sugar. Following overnight incubation at 37 °C, both the cultures were centrifuged at 8000 rpm for 5 min at 4 °C with successive washing of pellets washed with 0.85% saline. Hundred ml of double toned milk (Verka, Milk Plant in Ludhiana) was purchased from a local supermarket (Patiala, Punjab) and was heated to 80 °C in a water bath for 10 minutes and cooled to room temperature. Different concentrations of carrot juice (CY 10%, CY 15%, CY 20%), both with and without sugar was then added into it. One hundred ml of milk was inoculated with 1% inoculum (0.5% starter and 0.5% probiotic culture) in order to ferment milk to form yoghurt. The milk was fermented for 5-6 hrs to allow cells to reach exponential growth. After incubation, the samples were cooled at room temperature for approximately 1 hr and all samples were refrigerated (4±1°C). The experimental yoghurts were stored for 14 days and analyzed after 1, 7 and 14 days of storage. For Control, yoghurt from Nestle was used.

### **3.8 Sensory Evaluation, Storage and Viability of cultures**

Sensory qualities of the carrot yoghurt fermented by probiotic cultures along with control yoghurt were evaluated by a panel of judges by grading for colour, smell, consistency, texture and overall acceptability score on a 9-point hedonic scale (Moretti , 2004). Food samples were stored in sealed aseptic containers at 4 °C.

### **3.9 Analytical determination of yoghurt**

#### **3.9.1 Measurement of pH**

The pH of yoghurt was determined as described by Joseph *et al.* (2011) using bench-top pH meter which was calibrated with pH 7.0 and 4.0 standard buffers. On opening of the yoghurt, 25 ml of yoghurt was placed in a beaker, the probe was inserted and pH value was recorded.

#### **3.9.2 Syneresis**

The firmness of the yoghurt gel was measured as the susceptibility to syneresis based on the whey collected (amount of whey/100 gm of yoghurt) of freshly prepared (30 minutes refrigerated and ambient incubation) as well as after 14/21 days of refrigerated storage (Salwa *et al.*, 2013).

#### **3.9.3 Titratable acidity**

The titratable acidity was determined as described by Joseph *et al.* (2011) by titrating 15 ml of the yoghurt with 0.1 M sodium hydroxide until the yoghurt reached a pH value 8.2, corresponding to the end point of the phenolphthalein. The spent NaOH volume was recorded and the acid percentage of the yoghurt was calculated using the formula:

$$\text{Titratable acidity} = \frac{\text{Titre value} \times \text{M} \times 90 \times 100}{\text{Volume of sample} \times 1000}$$

### **3.10 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging assay**

The DPPH assay provided an easy and rapid way to determine the antioxidant activity of most of the substances. DPPH scavenging method was determined as described by Shetty *et al.* (1995). 250 µl yoghurt extracts were mixed with DPPH (3 ml of 60 mmol/L in ethanol). 250 µl of water

serves as control. The mixture was allowed to stand at room temperature for about 1 hr. Constant OD was recorded at 517 nm. The scavenging activity was calculated by:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} * 100$$

### 3.11 Hydroxyl radical scavenging activity

Hydroxyl-radical scavenging assay was used to assess the hydroxyl radical scavenging ability of the yoghurt extract. The reaction mixture consists of FeCl<sub>3</sub> (100µM), EDTA (104µM), H<sub>2</sub>O<sub>2</sub> (1mM) and 2-deoxy ribose (2.8mM) were mixed with or without extracts at the concentration of 9µM, the volume was made up to 1ml with phosphate buffer (20mM, pH 7.4). The reaction mixture was incubated at 37 °C in a water bath for 1hour. Then the reaction mixture was heated at 98 °C for 20 minutes after the addition of 1% TBA. The tubes were cooled which was followed by the addition of 1 ml of acetone to stabilize the colour. The absorbance was read at 535 nm. All the readings were corrected by including the appropriate controls. The control without any antioxidant or extract was considered as 100% deoxyribose oxidation. The percentage of hydroxyl radical scavenging activity of the extracts was determined accordingly in comparison with control (Mylarappa *et al.*, 2007)

### 3.12 Extraction and Quantification of Exopolysaccharide

Exopolysaccharides was extracted from Yoghurt according to method described by Uemura *et al.* (1998). One hundred gm of sample was diluted twice with distilled water i.e. 200 ml. Precipitation of Casein was done by adjusting the pH to 4.6 with 2M NaOH solution and then removing it with micro-organisms by centrifugation (10,000 g, 4 °C for 20 minutes). Whey fraction was neutralized by additional NaOH solution. Then the sample was heated for 30 minutes in boiling water bath. Insolubilized protein was removed by centrifugation (4 °C, 10,000 rpm, and 20 minutes). Equal volume of cold ethanol (99.5%) was added in the solution and was stirred at 4 °C overnight. The resulting precipitate was recovered by centrifugation. The solution was dissolved in distilled water. The precipitation procedure was repeated twice with ethanol and then again washed with distilled water. The sample was dialysed and then lyophilized.

Quantification was performed by the method described by Jana *et al* (2013). 5% phenol solution was dissolved in distilled water. 1 mg/ml glucose solution was prepared. Sample (400µl) was

dispensed into glass tube and 400µl of prepared phenol solution was added in it. For controlling, 400µl of 5% phenol solution in water was added in 400µl of distilled water. After that, concentrated Sulphuric acid (2ml) was added into the solution tube. Samples were stood for 10 minutes and then stirred and then again allowed to stand for 10 minutes at 30 °C. Sample was measured at 540 nm spectrophotometrically along with the control. The amount of EPS was calculated in milligram using calibration graph.

### **3.13 Evaluation of functional properties of the Carrot Yoghurt**

#### **3.13.1 Estimation of Ascorbic Acid**

The method of redox titration was followed using iodine solution (British Pharmacopeia 2003; Page 163, 164). The reaction proceeded with the oxidation of ascorbic acid by iodine to dehydroascorbic acid and conversion of iodine to iodide. Once all the ascorbic acid has been oxidized, the excess iodine is free to react with the starch indicator, forming the blue-black starch-iodine complex. This is the endpoint of the titration. To 10 ml aliquots of food extracts in 250 ml conical flasks, 4-5 drops of 1 % starch indicator was added. Titration was performed with 0.05 M iodine solution till the end point (blue-black colour, due to starch-iodine complex) was reached. The concentration of ascorbic acid present in the food extract was estimated from a standard plot where L-ascorbic acid was used in varying concentrations.

#### **3.13.2 Estimation of Riboflavin (Vitamin B<sub>2</sub>)**

Estimation of riboflavin was determined colorimetrically, 0.8 ml of the sample was added to 0.2 ml of 1 M Sodium Hydroxide. To 0.4 ml of the resulting solution, 1 ml of 0.1 M potassium phosphate buffer (pH 6) was added to neutralize. Absorbance at 444 nm was measured using a spectrophotometer. Concentration of riboflavin in the food samples was estimated with riboflavin as standard.

#### **3.14 Qualitative analysis of $\gamma$ -aminobutyric acid (GABA)**

GABA in the food extract was presumptively detected using Thin-Layer Chromatography (TLC) with some modifications as in the method described by Cho *et al.* (2007). Silica gel (30-40%) in distilled water was layered on clean glass plate using TLC applicator. Spotting was performed using 2 µl of GABA standard (GNC, USA) along with 20 µl of the food extract on the silica

plate. The elution solvent for TLC analysis was n-butanol, acetic acid, water in the ratio 4:1:1 which was allowed to run in a sealed glass chamber for 40 minutes. The solvent was evaporated in a hot air oven at 60 °C. The TLC plate was stained with ninhydrin solution and was incubated at 110 °C in a hot air oven for development of red-violet spots. The retention value,  $R_f$ , of the sample was compared with  $R_f$  of GABA which was 0.06.

$$R_f = \frac{\text{Distance travelled by component}}{\text{Distance travelled by Solvent}}$$

### 3.15 Quantitative Estimation of GABA

MRS broth containing overnight grown cultures was centrifuged at 15,000 rpm for 10 minutes and supernatant was taken. Culture supernatant (2µl) was added to 800 µl of methanol, and was incubated at 25°C in a water bath for 10 minutes. The reaction mixture was dried in incubator overnight and 1ml of 70mM LaCl<sub>3</sub> was added. Samples were then shaken for 15 minutes, centrifuged at 13000 rpm for 5 min, and 800 µl aliquots of supernatant was removed and placed in eppendorf tubes. Then 160 µl of 1M KOH was added and shaken for 5 minutes and centrifuged for 13,000 rpm for 5 min. The 1ml assay system contains 550 µl of supernatant, 200µl of 0.5 M K<sup>+</sup> pyrophosphate buffer (pH 8.6), 150 µl of 4mM NADP<sup>+</sup>, 50 µl of 2.5 units GABASE per ml, and 50µl of 20mM α-ketoglutarate. The initial absorbance was read at 340 nm before adding α-ketoglutarate, and the final absorbance was read after 60 minutes. The difference in the A<sub>340</sub> value was used to calculate GABA content in culture supernatant against GABA standard (Cho *et al.*, 2007).

### 3.16 Determination of β-Carotene in prepared Yoghurt

One gram portion of pureed carrot was used to which 10ml of acetone was added to it. The carrot was then crushed in pestle and mortar. Then sodium sulphate was added (pinch). The above formed solution is then transferred to the separating funnel. Then petroleum ether was added to the separating funnel. Two layers were formed in the separating funnel. Upper layer-petroleum ether which has beta- carotene, lower layer acetone which dissolves polar compounds. The upper layer of petroleum ether was taken. Then OD was recorded spectrophotometrically at 450 nm. The β-carotene content was calculated by:

$$\beta\text{-carotene} = \frac{\text{OD} * 13.9 * 10^4 * \text{volume of aqueous layer}}{\text{Weight of sample} * 560 * 1000}$$

### **3.17 Statistical Analysis**

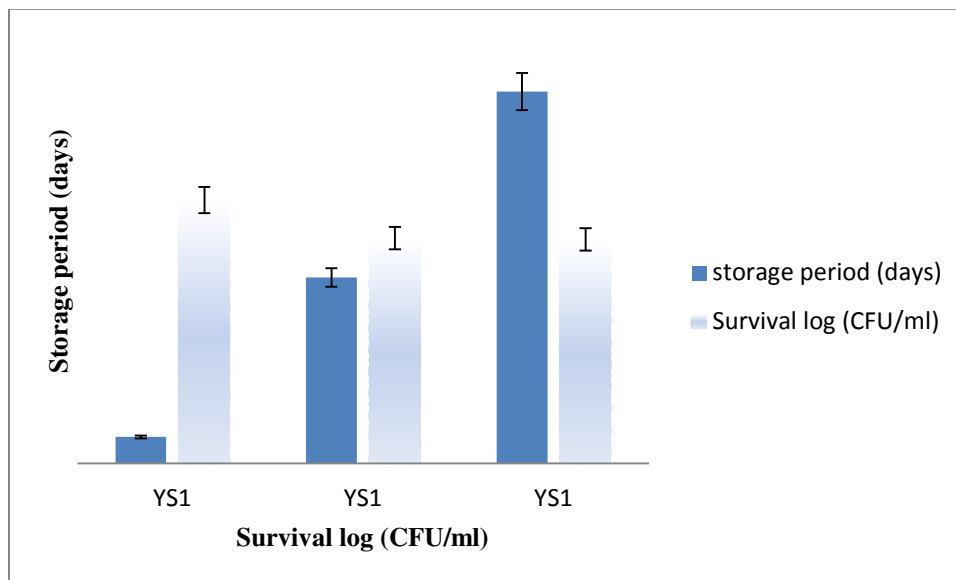
Data were expressed as mean±standard deviation (SD) from three independent parallel experiments. P < 0.05 was considered as statistically significant when compared to relevant controls.

# **RESULTS AND DISCUSSIONS**

### 4.1 Bacterial culture stability in Yoghurt

The yoghurt was stored overnight at 4 °C (approx. 24 hrs) exhibited a remarkable increase in the viable counts as compared to the counts after 7 and 14 days. No major inhibition was evident from the starter culture against *L. acidophilus*. This allows the co-existence of the starter culture with the adjunct culture. For the refrigerated cup of yoghurt, the total population of organisms in live and active culture yoghurt must be  $10^8$  cfu/g at the time of manufacture and in frozen yoghurt it must be at least  $10^7$  cfu/g at the time of manufacture (Chandan, 1999). In the present study, produced yoghurt showed that the viability of the cultures was retained over 14 days. No significant ( $p>0.05$ ) difference in viability over a period of 14 days were observed for both cultures.

Figure 5: Bacterial counts of the yoghurt



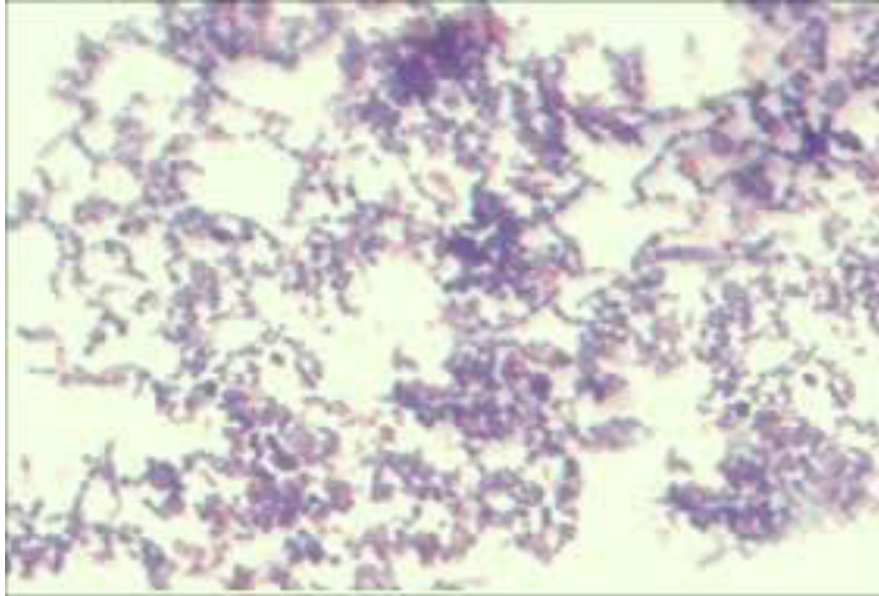


**Figure 6: Snapshot of 'Carrot Yoghurt' along with the label.**

## 4.2 Gram Staining

Gram staining revealed their morphology as gram positive, rod shaped, usually straight which appeared in pairs and short chains of varying length. Perhaps, gram staining of yoghurt revealed no counts of any contaminating micro-organisms.

**Figure 7: Gram Staining of *L. acidophilus* present in Carrot Yoghurt (20%)**



## 4.3 Evaluation of sensory attributes

**Table 3: Sensory evaluation of freshly prepared carrot yoghurt (CY) and control yoghurt with varying percentage of carrot juice**

Attributes	SAMPLES			
	Control	CY 10%	CY 15%	CY 20%
Smell (9)	5.8±0.02	6.6±0.02	7.0±0.01	7.6±0.03
Consistency (9)	6.1±0.02	6.6±0.02	7.2±0.05	7.5±0.05
Texture (9)	6.6±0.07	7.0±0.07	6.3±0.04	7.3±0.02
Color and appearance (9)	7.0±0.04	6.8±0.04	7.1±0.03	7.8±0.02
Overall Acceptability (9)	6.8±0.02	6.6±0.02	6.7±0.04	7.6±0.02

Data are represented as mean±SD (n=3)

**Table 4: Sensory evaluation of Carrot Yoghurt after 48 hrs of storage at 4 °C**

<b>AFTER 48 HRS STORAGE at 4 °C</b>	<b>SAMPLES</b>			
<b>Attributes</b>	<b>Control</b>	<b>CY 10%</b>	<b>CY15%</b>	<b>CY 20%</b>
Smell (9)	6.1±0.03	6.5±0.03	6.6±0.03	7.6±0.02
Consistency (9)	6.4±0.02	6.6±0.02	7.2±0.01	7.4±0.05
Texture (9)	6.1±0.06	6.5±0.06	6.2±0.02	7.2±0.04
Color and appearance (9)	5.9±0.03	6.4±0.03	6.8±0.06	7.6±0.07
Overall Acceptability (9)	6.2±0.04	6.3±0.04	6.7±0.05	7.4±0.06

Data are represented as mean±SD (n=3)

**Table 5: Sensory evaluation of Carrot Yoghurt after 13<sup>th</sup> day of storage at 4 °C**

<b>AFTER 48 HRS STORAGE at 4 °C</b>	<b>SAMPLES</b>			
<b>Attributes</b>	<b>Control</b>	<b>CY 10%</b>	<b>CY15%</b>	<b>CY 20%</b>
Smell (9)	5.1±0.03	6.1±0.03	6.0±0.03	7.4±0.02
Consistency (9)	5.9±0.02	6.0±0.02	7.0±0.01	7.2±0.05
Texture (9)	6.0±0.06	5.9±0.06	6.0±0.02	7.1±0.04
Color and appearance (9)	5.2±0.03	6.0±0.03	6.2±0.06	7.4±0.07
Overall Acceptability (9)	5.7±0.04	6.2±0.04	6.3±0.05	7.2±0.06

Data are represented as mean±SD (n=3)

The sensory scores revealed that the overall acceptability remained favourable for a sample CY 20% at 0<sup>th</sup>, 1<sup>st</sup> day (24 hrs) and 2<sup>nd</sup> day (48 hrs). Scores for other attributes are comparatively low when number of days increases. This can be attributed to the sweet taste of manufactured carrot yoghurt. After one week, yoghurt with 20% carrot juice showed better organoleptic properties as compared to the others. During the third week, yoghurt with Control yoghurt, 10% and 15% carrot juice were rejected as they spoiled rendering it unacceptable for consumption. However, yoghurt with 20% carrot juice can be considered acceptable, and showed better shelf life than others. The addition of 20% carrot juice to yoghurt showed to be the best concentration as it got the highest evaluation scores during the storage period. No unpleasant aftertaste, a pleasant level

of acidity and pleasing balance of flavor during the storage period. Keeping general acceptability in mind, the yoghurt having 20% carrot juice and storage of 24 hour was analyzed further.

#### 4.4 Analytical Determination of the sample

##### a. Changes in pH during yoghurt storage:

**Table 6: Changes in pH of the sample and control yoghurt with different time interval**

SAMPLE	pH of Yoghurt refrigerated at 4°C			
	0 Day	1 Day	7 Days	14 Days
Control Yoghurt	4.03±0.03	4.00±0.05	3.90±0.02	3.80±0.01
Sample Yoghurt	4.31±0.03	4.31±0.07	4.32±0.03	4.33±0.06

Data are represented as mean±SD (n=3)

Comparative study revealed that acidity of Control yoghurt increases during the refrigerated storage after 14 days which decreases the sensory properties of Control yoghurt as the sourness increases with time and the overall acceptability score for control yoghurt decreases as compared to Sample Yoghurt. This may be due to the fact that the yoghurt starter culture *S. thermophilus* is active even at refrigerated temperature and still can produce small amounts of lactic acid by fermentation of lactose which results in noticeable pH decrease (Shah *et al.*, 1995). The sample yoghurt having starter and adjunct culture remained almost stable even after 14 days. This may be due to the stability of *L. acidiphilus* at this pH. At low pH, *L. acidiphilus* produce enzymes that drive decarboxylation reactions (Sanders *et al.*, 1996). In this process, glutamate is an important amino acid which can be decarboxylated to help maintain pH. Glutamate decarboxylase (GAD) catalyses the decarboxylation of L-glutamate to glutamate-γ-aminobutyrate (GABA). *GadB*, the glutamate decarboxylase, converts internalized glutamate to GABA. *GadC* is involved in the antiport of glutamate and GABA. So, The acidresistance mechanism is glutamate-dependent.

##### b. Titratable acidity

The highest titratable acidity (0.80 % lactic acid) was observed in Sample yoghurt, while a lower titratable acidity of about 0.76 % was obtained in Control yoghurt. Previous studies have shown that fermentation of milk with probiotics incubated at 40–45 °C for a few hours decreases the pH

value to 3.9–4.3 and increases total titratable acidity for 0.64–0.97 % (Yang *et al.*, 2010). All of the samples produced in the present study were in accordance with the previous researches.

**Table 7: Titratable acidity of the sample and control yoghurt at refrigerated storage of 4 °C**

Sample	Titratable Acidity (%)
Control yoghurt	0.76±0.07
Sample yoghurt	0.80±0.05

Data are represented as mean±SD (n=3)

### c. Syneresis

Syneresis increased throughout the period of storage till 14 days. In Fresh yoghurt, lower syneresis in carrot yoghurt was observed. The syneresis in control was 15 ml while in Sample yoghurt, it was 3 ml which shows that the firmness of the yoghurt was maintained till 14th day. This may be due to previous reports that the yoghurt made using EPS-producing starter cultures had a lower level of syneresis than yoghurt produced with non-EPS-producing starter cultures (Amatayakul *et al.*, 2000).

**Table 8: Syneresis of the sample and control yoghurt during refrigerated storage at 4 °C**

Sample	Syneresis (ml)			
	0 Day	1 Day	7 Days	14 Days
Control	1±0.01	3±0.03	8±0.07	15±0.02
Sample yoghurt	0±0.02	2±0.02	3±0.06	3±0.01

Data are represented as mean±SD (n=3)

## 4.5 Estimation of Vitamin C and Riboflavin

Comparative study of Vitamin C and Riboflavin content in sample ‘yoghurt’ and that of control revealed slight increase in the concentration of Ascorbic acid in the test sample and a considerable improvement in riboflavin content from the control. Storage of the samples at 4 °C did not significantly alter the amount of Vitamin C and Riboflavin. There is evidence that the requirement for certain nutrients increase with ageing, for example vitamins C, B2, K and folate are some of the nutrients that have a protective role to play in the ageing process. Recent studies

concluded that LAB is increasingly gaining attention for elaborate production of micronutrients such as vitamins (Russo, 2012).

**Table 9: Vitamin C content of the sample and control yoghurt with increasing storage time**

Storage Period (hours)	Vitamin C content of sample 'yoghurt' (mg) for serving size of 100 grams	Vitamin C content of control 'yoghurt' (mg) for serving size of 100 grams	RDA* for elderly
0	267.61	110.43	90-120 mg/day
24	266.80	109.56	
48	266.32	109.23	

RDA: Recommended Dietary Allowance Source: Food and Nutrition Board, Institute of Medicine, National Academies (USA)

**Table 10: Vitamin B<sub>2</sub> content of the sample and control yoghurt with increasing storage time**

Storage Period (hours)	Vitamin B <sub>2</sub> content of sample 'yoghurt' (mg) for serving size of 100 grams	Vitamin B <sub>2</sub> content of control 'yoghurt' (mg) for serving size of 100 grams	RDA* for elderly
0	33.68	15.55	1.2-1.6 mg/day
24	33.33	15.12	
48	32.67	14.78	

RDA: Recommended Dietary Allowance Source: Food and Nutrition Board, Institute of Medicine, National Academies (USA)

#### 4.6 Estimation of Beta-Carotene

Biochemically, carrot is a rich source of  $\beta$ -carotene, fiber and many essential micronutrients and functional ingredients. Comparative study of beta-carotene content in sample 'yoghurt' and that of control revealed significant ( $p < 0.05$ ) increase in the concentration of  $\beta$ -carotene in the test sample. High concentrations of carotenoids, especially  $\beta$ -carotene in carrots make them to inhibit cancers, free radical scavengers, anti-mutagenic and immune-enhancers (Karki *et al.*, 2012). Therefore, it may enhance longevity and decrease the onset of chronic diseases in humans. It has been shown that if subjects consume fat soluble components that are not absorbable or absorbable only to a limited extent, their plasma carotenoid concentrations may decrease

substantially (Karin et al., 2000). This indicates that the carrot yoghurt meets the requirement of geriatric population in one serving.

**Table 11: Beta-carotene content of the sample and the control yoghurt with increasing storage time**

Storage Period (hours)	$\beta$ -Carotene content of sample 'yoghurt' ( $\mu$ g) for serving size of 100 grams	$\beta$ -Carotene content of control 'yoghurt' ( $\mu$ g) for serving size of 100 grams	RDA* for elderly
0	5130.12	1120.56	390-18000 $\mu$ g/day
24	5128.34	1120.43	
48	5128.23	1119.32	

RDA: Recommended Dietary Allowance Source: Food and Nutrition Board, Institute of Medicine, National Academies (USA)

#### 4.7 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect

**Table 12: DPPH radical scavenging activity of different Yoghurt samples.**

Sample	% DPPH Radical Scavenging Activity
Negative Control (water)	21.3% $\pm$ 1.5
Control yoghurt	43.3% $\pm$ 1.5
Sample yoghurt	88.6% $\pm$ 1.7

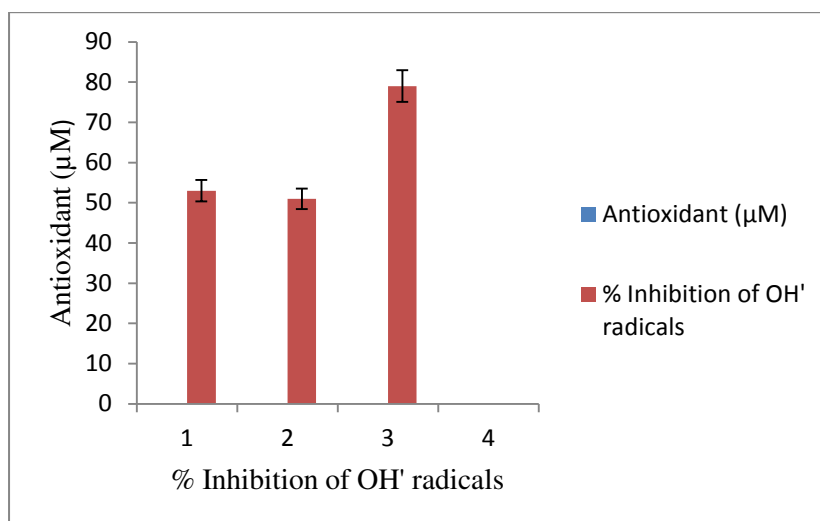
Data are represented as mean $\pm$ SD (n=3)

Sample yoghurt exhibited high DPPH radical scavenging activity of 88.6% while control Yoghurt showed the lowest DPPH inhibition. Higher yoghurt antioxidant activity in the presence of *L. acidophilus* is beneficial in two respects, firstly to delay the oxidation process of lipids in yoghurt that are responsible for the formation of off-flavours and undesirable chemical compounds (Berset *et al.*, 1994) and secondly, to increase dietary antioxidants which are crucial in preventing the progressive impairment of pancreatic beta-cell function due to oxidative stress (Liu *et al.*, 2005). The results indicate that the developed yoghurt is a powerful free radical scavenger

#### 4.8 Hydroxyl radical scavenging activity

Hydroxyl radicals are known to be the most reactive of all the reduce forms of dioxygen and are thought to initiate cell damage in vivo. The yoghurt EPS scavenges the hydroxyl radicals up to the 79% at 10 $\mu$ M concentration, whereas standard at 400 $\mu$ M scavenged 52-55%.

**Figure 8: Hydroxyl radicals scavenging activity of Yoghurt**



#### 4.9 Quantification of Exopolysaccharide

**Table 13: EPS contents of control and sample yoghurt**

Sample	EPS (g/100 gm)
Control yoghurt	0.979±0.04
Sample yoghurt	4.40±0.03

Data are represented as mean±SD (n=3)

Exopolysaccharide production of the LAB is an important attribute for the fermented dairy products. The results showed that sample yoghurt produced greater quantities of EPS (4.4 mg/100 gm), as compared to control yoghurt. Significant ( $p < 0.05$ ) difference obtained in production of EPS by control and sample yoghurt. Also, when compared with the literature (Shihata, 2002), the amount of EPS production was above the amount given in the literature. Amounts of EPS from *S.thermophilus* can range from 30 mg/ L (Cerning, 1988) to 890mg /L (Escalante *et al.*, 1998). The composition of medium may be responsible for the yield

of EPS. This results in good texture, consistency and stability of the yoghurt because EPS has texture promoting abilities and high water binding.

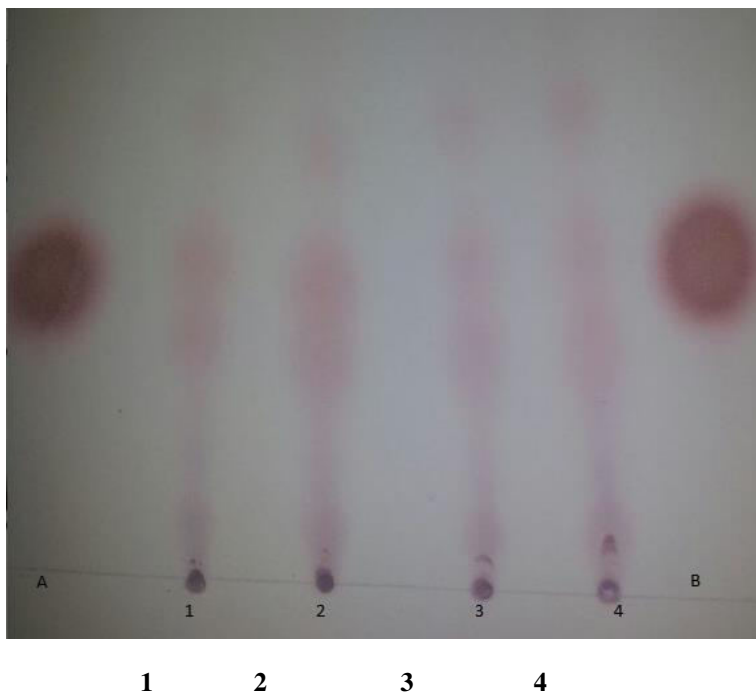
#### 4.10 Qualitative determination of GABA

The identification of GABA was done on the basis of Retention Factor ( $R_f=0.63$ ) with authentic GABA samples. A and B are the GABA standards, while 1, 2, 3 and 4 are the yoghurt samples. Since, it is well known that GABA plays an important role in central nervous system as an inhibitory neurotransmitter (Pradeep *et al.*, 2011). It also rejuvenates the memory and regulates blood sugar level. Therefore, the amount of GABA incorporated into the yoghurt, it seems, is high enough to have some functional value. The Lactobacillus strain and other GABA producing lactic acid bacteria seem to show a prospect to be applied in dairy and other health products that can exploit the functional properties of GABA.

#### 4.11 Quantification of GABA production in Carrot Yoghurt (20%)

The GABA concentration (mM) in Carrot Yoghurt was found to be 6.08mM/g. This satisfies the GABA requirement for alleviation of stress, hypertension through food.

**Figure 9: TLC chromatogram of standard GABA and yoghurt sample.**



So far, few studies have reported the production of GABA by *S. thermophilus* or *L. acidophilus*. GABA acts as the major inhibitory neurotransmitter in the mammalian central nervous system and shows well known physiological functions: neurotransmission, induction of hypotension, and diuretic and tranquilizer effects (Wong *et al.*, 2003; Jacobs *et al.*, 1993). Incorporation of GABA, having tranquillizing and hypotensive properties, in diet has been an established remedy for stress, a major risk factor in coronary heart disease. GABA also exerted positive effects for treatment of sleeplessness, depression, and autonomic disorders (Okada *et al.*, 2000), chronic alcohol related symptoms (Oh *et al.*, 2003) and stimulation of immune cells. Therefore, GABA enriched yoghurt may help in proper brain functioning and alleviate stress in Geriatric subjects.

Overall, the results of our study indicates that incorporation of Carrot Juice in Yoghurt produced by using *S. thermophilus* and *L. acidophilus* imparts high nutraceutical benefits in terms of Vitamin C, Riboflavin and Calcium in addition to  $\beta$ -carotene. These attributes are important in addressing the nutritional deficiencies in the elderly. Therefore, the developed yoghurt is apt for the geriatric population (in addition to other age groups). However, clinical trials are recommended to commercialize it.

# **CONCLUSION**

## Chapter 5. CONCLUSION

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The main objective of the study was to develop a functional fermented food with a wide range of bioactive compounds. The results of this study showed that the use of carrot juice in yoghurt was advantageous as it can deliver increased amount of the  $\beta$ -carotene content to the Geriatric population. Also, carrot is used as vitaminized food supplement. . The yoghurt sample supplemented with Carrot Juice (20%) and was the best formulation for yoghurt sensorially.

Exopolysaccharides (EPS) from lactic acid bacteria (LAB) have found their most valuable application in the improvement of the texture properties of fermented milk products as well yoghurt and have been studied extensively over the past decades. Their production significantly contributes to texture, mouth feel, taste perception and stability of the final products. There is a high consumer demand for products with low fat or sugar content and low levels of food additives especially stabilizers and thickeners, as well as cost factors, make EPS a viable alternative. High levels of EPS were observed in the CY (20%).

Nutrient deficiencies (Vitamin C, Vitamin B2, and Calcium) are often common in elderly people due to a number of factors such as their reduced food intake and a lack of variety in the foods consumed. Vitamin C (267.31  $\mu\text{g}/100\text{gm}$ ), Riboflavin (33.68  $\mu\text{g}/100\text{gm}$ ) concentration was significant in the developed Carrot Yoghurt (20%).

The yoghurt addresses most of the functional properties suitable for Indian Geriatric population. It is also acceptable to other age groups (women and children) in terms of addressing nutritional deficiencies (Riboflavin,  $\beta$ -carotene and Vitamin C). The developed yoghurt has shelf life of 14 days at 4 °C without the loss of colour and organoleptic properties and is suitable for commercial purpose. However, clinical studies are recommended prior to application of food.

## Chapter 6. PARTICIPATION ON CONFERENCE

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Awarded 1<sup>st</sup> Prize in Poster Presentation at DBT, ICMR and DST sponsored 4<sup>th</sup> International Conference on Updating Food Technology: A Challenge Towards Public Health Nutrition ICUFT-2014, organized by IIFANS, JNU, New Delhi, May 2014

ICMR & DST Sponsored 4<sup>th</sup> International Conference on Updating Food Technology : A Challenge towards Public Health Nutrition (ICUFT-2014)

### PHN-P-02

#### **DEVELOPMENT OF GERIATRIC FOOD AND ITS FUNCTIONAL POTENTIAL**

Jhilmil Tandon, Arkadeep Mukherjee and Abhijit Ganguli

Department of Biotechnology, Thapar University, Patiala.

Nutrition is an important determinant of health in persons over the age of 55. Malnutrition in the elderly is often under diagnosed. The decline in physiological and health functions makes the elderly develop some kinds of diseases. *Streptococcus thermophilus* RDT, a yoghurt bacteria as well as probiotic culture has been suggested for various forms of Geriatric deficiencies. The study was designed to explore the potential of *Streptococcus thermophilus* as yoghurt starter culture. Plain yoghurt was prepared in laboratory with optimized conditions of sodium alginate (1%) and inoculum (1%). The sensory, chemical and microbiological quality of yoghurt samples were investigated during refrigerated storage at 4o C for two weeks. In comparison to commercially available yoghurt, the titratable acidity, pH, syneresis, stability of bacteria and shelf life was found more significant. Presence of Gamma-Aminobutyric acid (GABA) was also confirmed. The firmness improved with the addition of *Streptococcus thermophilus*. Exopolysaccharide (EPS) remain an interesting tool to modulate the sensory properties and stability of yoghurt. Extraction and quantification of Exopolysaccharide was performed. Production of EPS was quite significant (0.7979 mg/100gm of yoghurt). Nutritional analysis was also conducted in yoghurt sample. Presence of riboflavin, Calcium and Vitamin C was studied which satisfied the Recommended Dietary Allowance of Geriatric population. These results were encouraging and further large scale studies seem justified to establish the place of probiotic supplements in elderly subjects. Keywords: *Streptococcus thermophilus*, Geriatric, Exopolysaccharide, Yoghurt.



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# REFERENCES

## Chapter 7. REFERENCES

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Agabriel C, Cornu A, Journal C, Sibra P, Grolier G and Martin B. Tanker milk variability according to farm feeding practices: Vitamins A and E, carotenoids, color, and terpenoids. *Journal of Dairy Science*. 2007; 90: 4884–4896.

Agarwal K N, Bhasin S K, Faridi M M, Mathur M, Gupta S. *Lactobacillus casei* in the control of acute diarrhea-a pilot study. *Indian Pediatrics*. 2001; 8: 905-10.

Ali F S, Saad A O, Salwa A G. Probiotic stability of yoghurts during refrigerated storage. *Journal of Biotechnology*. 2013; 5(2): 9-19.

Amatayakul T, *et al.* Physical characteristics of yoghurts made using exopolysaccharideproducing starter cultures and varying casein to whey protein ratios. *Nutritional Biochemistry*. 2000: 9: 431-435.

Beom J, Yong S, Myung H. Antioxidant activity of vegetables and blends in iron catalyzed model system. *Journal of Food Science and Nutrition*. 1998; 3:309–314.

Bhanwar S, Bamnia M, Ghosh M and Ganguli A. Use of *Lactococcus lactis* to enrich sourdough bread with  $\gamma$ -aminobutyric acid. *International Journal of Food Science and Nutrition*. 2013; 64:77-81.

Brand-Williams W, Cuvelier M E, Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebenson Wiss Technology*. 1995; 28:25-30.

Brody Tom. *Nutritional Biochemistry*. San Diego: Academic Press. 1999.

Cahill L, Corey P N, Sohemly A. Vitamin C deficiency in a population of Young Canadian Adults. *American Journal of Epidemiology*. 2009; 170: 464-471.

Cakmakc S, Cetin B, Turgut T, Gurses M and Erdogan . Probiotic properties, sensory qualities, and storage stability of probiotic banana yoghurts. *Turkish Journal of Veterinary Animal Sciences*. 2012; 36: 231–237.

Cerning J, Bouillanne C, Desmazeaud M J & Landon M. Exocellular polysaccharide production by *Streptococcus thermophilus*. *Biotechnology Letters*. 1988; 10: 255–260.

- Cerning J, Bouillanne C, Desmazeaud M J, & Landon M. Isolation and characterization of exocellular polysaccharide produced by *Lactobacillus bulgaricus*. *Biotechnology Letters*. 1986; 8: 625–628.
- Chandan R C. Enhancing market Value of Milk by Adding Cultures. *Journal of Dairy Science*. 1999; 82:2245-2256.
- Cho Y R, Chang J Y, Chang H C. Production of gamma aminobutyric acid (GABA) by *Lactobacillus buchneri* isolated from kimchi and its neuroprotective effect on neuronal cells. *Journal Microbiology and Biotechnology*. 2007; 17: 104-109.
- Claesson M J, Jeffery I B, *et al*. Gut microbiota composition correlates with diet and health in the elderly. *Nature*. 2012; 488: 178-183.
- DeVerse M, Keller, and Barth C A. Enhancement of intestinal hydrolysis of lactose by microbial  $\beta$  galactosidase (EC 3.2.1.23) of kefir. *British Journal of Nutrition*. 1992; 67: 67-75.
- Escalante A, Wacher-Rodarte C, Garcia-Garlbay M & Farres A. Enzymes involved in carbohydrate metabolism and their role on exopolysaccharide production in *Streptococcus thermophilus*. *Journal of Applied Microbiology*. 1998; 84: 108–114.
- Fernandes C F, Shahani, K M, and Amer M A. 'Therapeutic role of dietary lactobacilli and lactobacillic fermented dairy products'. *FEMS Microbiology. review*. 1987; 466: 343-356.
- Franceschi C *et al*. Inflamm-aging: an evolutionary perspective on immunosenescence. *Annals of the New York Academy of Sciences*. 2000; 908: 244–254.
- Fuller, Gibson G R. aspect in vitro and in vivo research approaches directed toward identifying prebiotics and probiotics for human use. *Journal of Nutrition*. 2000; 130: 391S-395S.
- Gassem M A, Schmidt K A & Frank J F. Exopolysaccharide production in different media by lactic acid bacteria. *Cultured Dairy Products Journal*. 1995; 30: 18–21.
- Goldin, B R & Gorbach, S L. The effect of milk and lactobacillus feeding on human intestinal bacterial enzyme activity. *American Journal of Clinical Nutrition*. 1984; 39(5): 756-761.

Gopal K, Nath A. Geriatric health in India: Concerns and Solutions. *Indian Journal of Community Medicine*. 2008; 33: 214-218.

Griffin A M, Morris V J, & Gasson M J. The cpsABCDE genes involved in polysaccharide production in *Streptococcus salivarius* subsp. *thermophilus* strain NCBF 2393. *Gene*. 1993; 183: 23–27.

Guandilini S, *et al.* *Lactobacillus* GG administered in oral rehydration solution to children with acute diarrhoea: A multicentre European trial. *Journal of paediatric gastroenterology and Nutrition*. 2000; 30: 54-60.

Guigoz Y, Dore J & Schiffrin E J. The inflammatory status of old age can be nurtured from the intestinal environment. *Current opinion in clinical nutrition and metabolic care*. 2008;11: 13–20.

Gursoy, *et al.* Set Type Yoghurt Production by Exopolysaccharide Producing Turkish Origin Domestic Strains of *Streptococcus thermophilus* (W22) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (B3). *Kafkas Univitesisi Veteriner Fakultesi Dergisi*. 2010; 16 (Suppl-A): S81-S86.

Hayakawa K, Kimura M, Kasaha K, Matsumoto K, Sansawa H & Yamori Y. Effect of a  $\gamma$ -aminobutyric acid-enriched dairy product on the blood pressure of spontaneously hypertensive and normotensive Wistar–Kyoto rats. *British Journal of Nutrition*. 2004; 92: 411–417.

Hess S J, Roberts R F, & Ziegler G R. Rheological properties of nonfat yoghurt stabilized using *Lactobacillus delbrueckii* subsp. *bulgaricus* producing exopolysaccharide or using commercial stabilizer systems. *Journal of Dairy Science*. 1997; 80: 252–263.

Inoue K, Shirai T, Ochiai H, Kasao M, Hayakawa K, Kimura M & Sansawa H. Blood-pressure-lowering effect of a novel fermented milk containing  $\gamma$ -aminobutyric acid (GABA) in mild hypertensives. *European Journal of Clinical Nutrition*. 2003; 57: 490–495.

Jakobs C, Jaeken J & Gibson K M. Inherited disorders of GABA metabolism. *Journal of Inherited Metabolic Disease*. 1993; 16: 704–715.

Jana, *et al.* Potential of Exopolysaccharides in Yoghurt Production. *International Journal of Agricultural Biosystems Science and Engineering*. 2013; 7: 75-78.

Joseph A O, Olugbuyiro and Joy E O. Physico-chemical and Sensory Evaluation of Market Yoghurt in Nigeria. *Pakistan Journal of Nutrition*. 2011; 10 (10): 914-918.

Karagul Y, Wilson C and White H. Formulation and processing of yoghurt. *Journal of Dairy Science*. 2004; 87: 543- 550.

Karin H, *et al*. Dietary Factors That Affect the Bioavailability of Carotenoids. *Journal of Nutrition*. 2000; 22: 503-506.

Knockaert G, *et al*. Changes in  $\beta$ -carotene bioaccessibility and concentration during processing of carrot puree. *Food Chemistry*. 2012; 133: 60–67.

Krogsgaard-Larsen P, Williams M, Glennon R A, Timmermans P M W M. GABA receptors. In: Receptor Pharmacology and Function. *Marcel Dekker Inc, New York*. 1989; pp. 349–383.

Lankaputhra W E V and Shah N P. Antimutagenic properties of probiotic bacteria and of organic acids. *Mutation Research*. 1998; 397: 169-182.

Liu D Z, Lin Y S, Hou W C. Monohydroxamates of aspartic acid and glutamic acid exhibit antioxidant and angiotensin converting enzyme inhibitory activities. *Journal of Agricultural and Food Chemistry*. 2005; 52: 2386–2390.

Lu X, Chen Z, Gu Z & Han Y. Isolation of  $\gamma$ -aminobutyric acid-producing bacteria and optimization of fermentative medium. *Biochemical Engineering Journal*. 2009; 41: 48–52.

Manyam B V, Katz L, Hare, T A, Kaniefski K & Tremblay R D. Isoniazid-induced elevation of cerebrospinal fluid (CSF) GABA levels and effects on chorea in Huntington's disease. *Annals of Neurology*. 1981; 10: 35–37.

Mayer R, Cherry J & Rhodes D. Effect of heat shock on amino acid metabolism of cowpea cells. *Plant Physiology*. 1990;94: 796–810.

McKinley M C. The nutrition and health benefits of yoghurt. *International Journal of Dairy Technology*. 2005; 58: 1–12.

Mody I, Dekoninck Y, Otis T S, Soltesz I. Bringing the cleft at GABA synapse in the brain. *Trends Neurosciences*. 1994; 17: 517–525.

- Moretti V M, Madonia G, Diaferia C, Men-Tasti T, Paleari M A, Panseri S, Pirone G, Gandini G. Chemical and microbiological parameters and sensory attributes of a typical Sicilian salami ripened in different conditions. *Meat Science*. 2004; 66: 845-854.
- Morley E. The Elderly Type 2 Diabetic Patient: Special Considerations. *Diabetic Medicine*. 1998; 15 (Suppl. 4): S41–S46.
- Mylarappa *et al.* Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts. *Food Chemistry*. 2008; 106: 720–728.
- Nakajima H, Toyoda S, Toba T, Itoh T, Mukai T, Kitazawa H & Adachi S. A novel phosphopolysaccharide from slime-forming *Lactococcus lactis* subspecies *cremoris* SBT 0945. *Journal of Dairy Science* 1990; 73: 1472–1477.
- Noh D O, Kim S H and Gilliland S E. Incorporation of cholesterol into the cellular membrane of *Lactobacillus acidophilus* ATCC 43121. *Journal of Dairy Science*. 1997; 80: 3107- 3113.
- Nomura M, Kimoto H, Someya Y, Furukawa S & Suzuki I. Production of  $\gamma$ -aminobutyric acid by cheese starters during cheese ripening. *Journal of Dairy Science*. 1998; 81: 1486–1491.
- Padayatty S J, Katz A, Wang Y, Eck P, Kwon O, *et al.* Vitamin C as antioxidant: evaluation of its role in disease prevention. *Journal of the American College of Nutrition*. 2003; 22: 18-35.
- Parker R B. Probiotics, the other half of the antibiotic story. *Animal Nutrition and Health*. 1974; 29:4–8.
- Perdigeon G, Valdez J and Rachid M. Antitumour activity of yoghurt. Study of possible immune response. *Journal of Dairy Research*. 1998; 65: 129-138.
- Rampelli S, Candela M, Donini L, *et al.* A probiotics containing biscuit modulates the intestinal microbiota in the elderly. *Journal of Nutrition Health and Ageing*. 2013; 17: 166-170.
- Rao C V, Sanders M, Indranie C, Reddy B S. Prevention of colonic preneoplastic lesions by the probiotic *Lactobacillus acidophilus* NCFM in F344 rats. *International Journal of Oncology*. 1999; 14:939-944
- Ring E, Gatesoupe F J. Lactic acid bacteria in Fish: a review. *Aquaculture*. 1998; 160:177-203.

- Russo P, *et al.* Beta glucans improve growth, viability and colonization of probiotic microorganisms. *International Journal of Molecular Science*. 2012; 13: 6026-6039.
- Sanders J W, *et al.* A chloride-inducible acid resistance mechanism in *Lactococcus lactis* and its regulation. *Molecular Microbiology*. 27:299–310.
- Saunier K, Dore J. Gastrointestinal tract and the elderly: functional foods, gut microflora and healthy ageing. *Digestive and Liver Diseases*. 2002; 34: S19-S24.
- Schieber A, Marx M and Carle R. Simultaneous determination of carotenes tocopherol in ATBC drinks by high-performance liquid chromatography. *Food Chemistry*. 2002; 76: 357-362.
- Sharma K D, Karki S, Thakur N S, Attri S. Chemical composition, functional properties and processing of carrot. *Journal of Food Science and Technology*. 2012; 49(1): 22-32.
- Sharma K, Karki S. Chemical composition, functional properties and processing of carrot. *Journal of Food Science and Technology*. 2011; 49(1): 22-32.
- Shetty, *et al.* Pre-vention of verification associated with in vitro shoot culture of oregano (*Origanum vulgare*) by *Pseudomonas* spp. *Journal of Plant Physiology*. 1995; 147: 447–451.
- Shihata A, Shah N P. Influence of addition of proteolytic strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* to commercial ABT starter cultures on texture of yoghurt, exopolysaccharide production and survival of bacteria. *International Dairy Journal*. 2002; 12: 765–772.
- Simova E D, Frengova G T and Beshkova E. Synthesis of carotenoids by rhodotorula rubra cultured with yoghurt starter whey ultra filtrate. *Journal of the Society of Dairy Technology*. 2004; 31: 115-121.
- Singh *et al.* Survival of Probiotic Strains in Non-dairy Indian Spice Condiment Exhibiting Cholesterol Reducing Properties. *Food Science and Biotechnology*. 2012; 21(5): 1309-1315.
- Sun M S, Mihyang K, Song J B. Cytotoxicity and quinine reductase induced effects of Daucus carrot leaf extracts on human cells. *Korean Food Science*. 2001; 30:86–91.

Sun T, Zhao S, Cai C, Chen Y & Zhang H. ACEinhibitoryactivity and gamma-aminobutyric acid content of fermented skim milk by *Lactobacillus helveticus* isolated from Xinjiang koumiss in China. *European Food Research and Technology*. 2009; 228: 607–612.

Tamime A Y & Deeth H C. Yoghurt: Technology and biochemistry. *Journal of Food Protection*. 1980; 43: 939–977.

Torheim L E, Ferguson E L, Penrose K, Arimond M. Women in resource-poor settings are at risk of inadequate intakes of multiple micronutritents. *Journal of Nutrition*. 2010; 140: 2051S-2058S.

Uemura J, *et al.* Chemical characterization of exocellular polysaccharide from *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1073R-1. *Milchwissenschaft*. 1998; 53: 443–446.

Verschuere L Rombaut G, Sorgeloos P Verstraete W. Prbiotic bacteria as biological controlagents in aquaculture. *Microbiology and Molecular Biology Review*. 2000; 64: 655-671.

Wacher-Rodarte, *et al.* Yoghurt production from reconstituted skim milk powder using different polymer and non-polymer forming starter cultures. *Journal of Dairy Research*. 1993; 60: 247–254.

Wayne S J, Rhyne R L, Garry P J and Goodwin JS. Cell-mediated immunity as a predictor of morbidity and mortality in the aged. *The Journals of Gerontology, Series A: Biological Sciences*. 1990; 45:M45–8.

Yang M, *et al.* Physicochemical, Textural and Sensory Characteristics of Probiotic Soy Yoghurt Prepared from Germinated Soybean. *Food Technology and Biotechnology*. 2010; 48 (4): 490–496.

Yeun P S M, Sanders M E, Kitts C L, Cano R, Tong P S. Species-specific identification of commercial probiotic strains. *Journal of Dairy Science*. 2011; 85: 139–1051.

Zulueta A, Barba F J, Esteve M J and FriGola A. Effects on the carotenoid pattern and vitamin A of a pulsed electric field-treated orange juice–milk beverage and behavior during storage. *European Food Research and Technology*. 2010; 231: 525–53

**MRS (De Man, Rogosa and Sharpe) broth**

<b>Ingredients</b>	<b>Quantity (g/L)</b>
Peptone from casein	10.0
Beef extract	10.0
Yeast extract	5.0
Dextrose	20.0
Dipotassium hydrogen phosphate	2.0
Tween 80	1.0
Triammonium citrate	2.0
Sodium acetate	5.0
Magnesium sulphate	0.2
Magnesium sulphate	0.04

pH adjusted with 10N NaOH to pH 7.0 before autoclaving. Sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes.

**MRS agar:** MRS broth containing 15.0 g/L agar.

**Streptococcus thermophilus Isolation Agar**

<b>Ingredients</b>	<b>Quantity (g/L)</b>
Casein enzymatic hydrolysate	10.0
Yeast extract	5.0
Sucrose	10.0
Dipotassium phosphate	2.0
Agar	15.0

Final pH 6.8 +/- 0.2 at 25 °C

## **BUFFERS AND SOLUTIONS**

### **1. 0.1M Phosphate buffer pH 7**

Monobasic sodium phosphate, monohydrate (1 M)      61.5

Dibasic sodium phosphate, monohydrate (1 M)      38.5

### **2. Iodine solution (0.05 M)**

0.332 gm of Iodine and 0.507 gm Potassium Iodate are dissolved in 200 ml distilled water

### **3. Starch Indicator solution (1%)**

0.5 g of soluble starch added to 50 ml of near boiling water. Stirred to dissolve and cooled before use

### **4. Ninhydrin Solution**

20 g of ninhydrin in 600 ml of ethanol

### **5. Phenol Solution (5%)**

5g of fresh phenol added in 100 ml distilled water

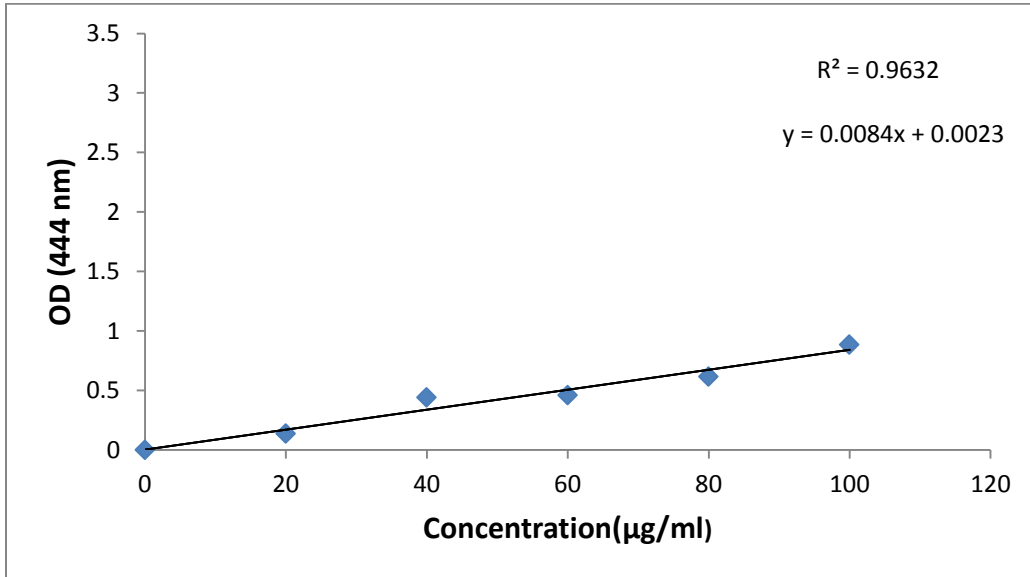
### **6. NaOH Solution (0.1 M)**

0.4 g of NaOH in 100 ml of distilled water

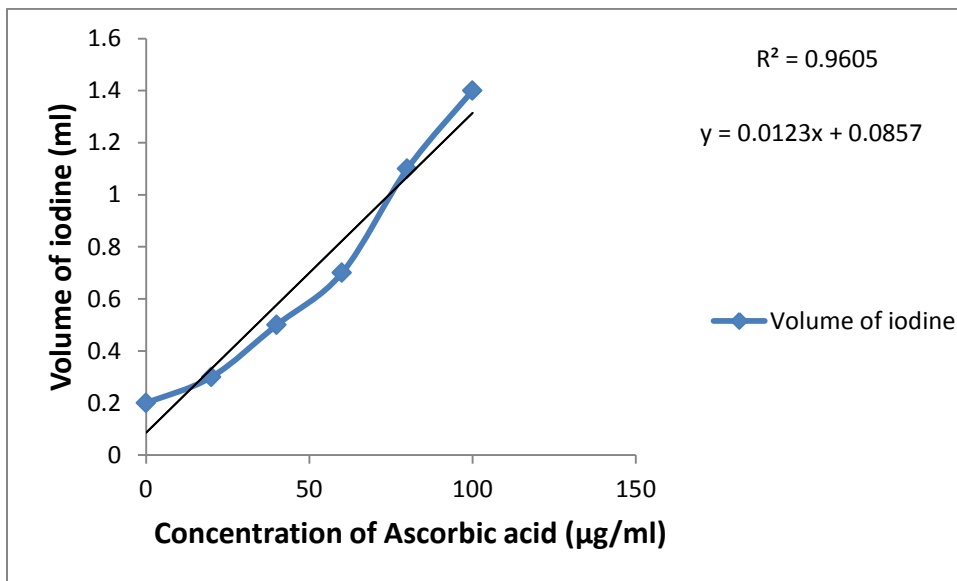
### **7. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH)**

Dissolve 3 ml of 60 mmol DPPH in 1L distilled water.

1. Standard Curve for Riboflavin estimation.



2. Standard Curve for Vitamin C estimation.



### 3. Standard curve of Glucose for Quantification of Exopolysaccharide.

